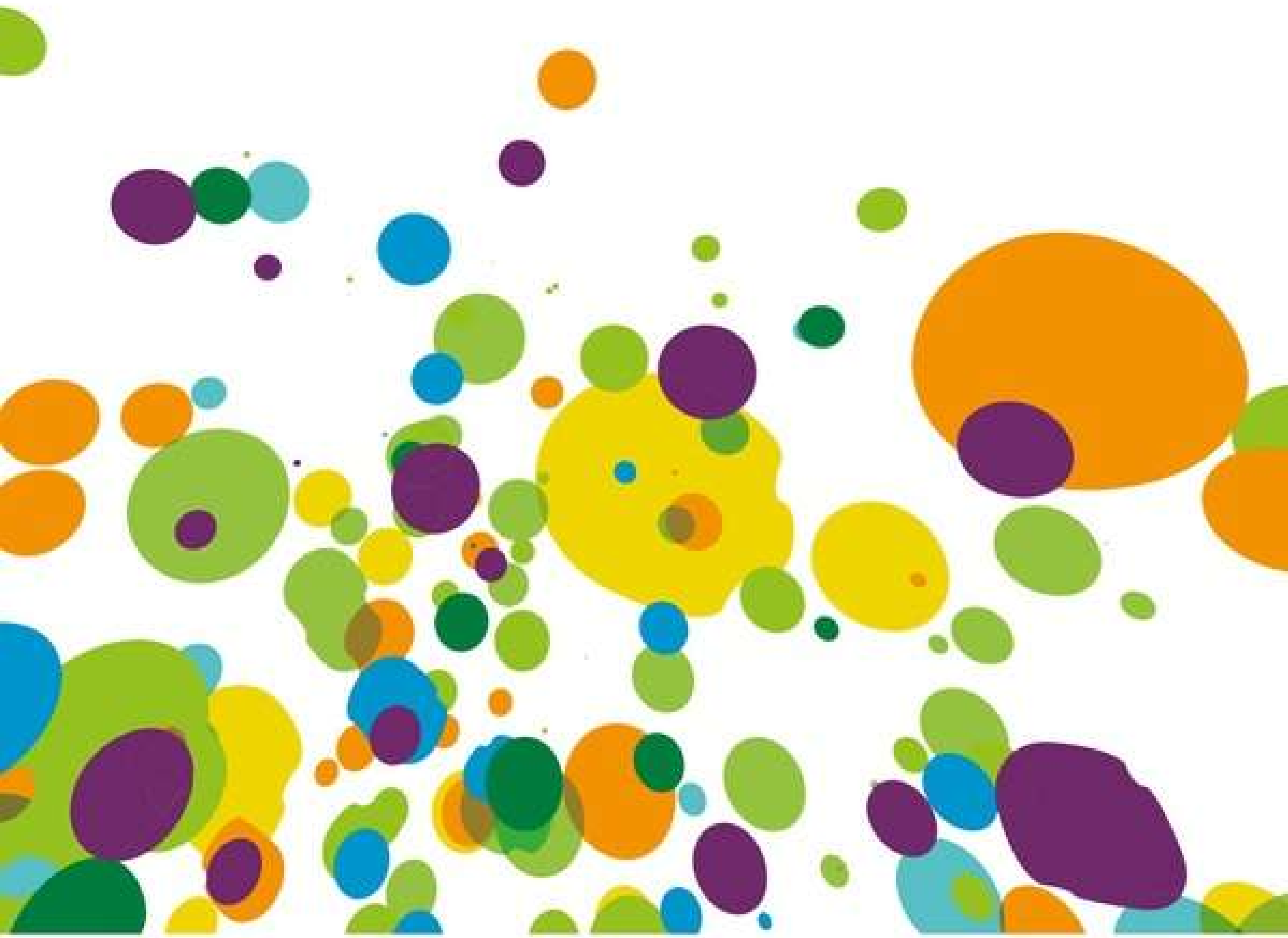
ENDOCRINE FRAILTY IN THE ELDERLY

EDITED BY: Sandro La Vignera, Antonio Aversa and Fabio Monzani
PUBLISHED IN: Frontiers in Endocrinology



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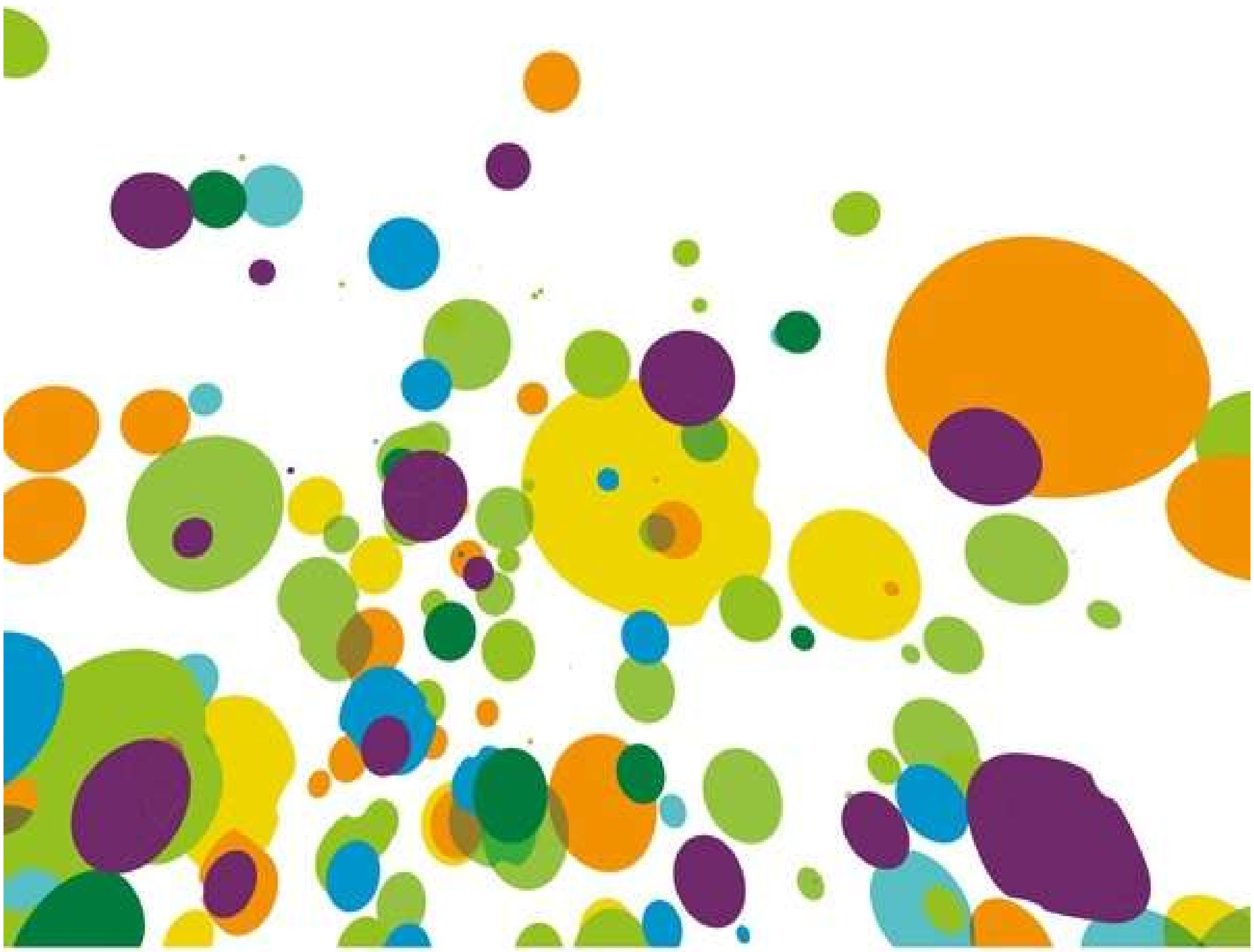

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ENDOCRINE FRAILTY IN THE ELDERLY

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Editorial: Endocrine Frailty in the Elderly

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Keywords: endocrine frailty, osteoporosis, hypogonadism, diabetes, thyroid disorders

Editorial on the Research Topic
Endocrine Frailty in the Elderly

Geriatric Endocrinology represents a challenge for each of the two specialists involved. Accordingly, malpractice is common since the change of the hormone balance with aging and the presence of comorbidities. Frailty is a multifactorial clinical entity with a complex physiopathology, characterized by alterations of several functional pathways from an endocrinological point of view. The present issue of Frontiers in Endocrinology focuses on the most recent advances in the field of endocrinology of aging, particularly referring to endocrine-related frailties. The aim is to evaluate the main endocrinological changes of the elderly and their systemic clinical relapses. The structure of the special issue includes 11 reviews and two original articles, dealing with the main chapters, and hot topics of geriatric endocrinology.

Longo et al. deeply discuss the clinical management of type 2 diabetes, which represents a real challenge for the physician. They also undertake the management of glycemic goals and antihyperglycemic treatments in accordance to the medical history and comorbidities, giving preference to drugs that are associated with low risk of hypoglycemia (i.e., metformin, pioglitazone, dipeptidyl-peptidase-4 inhibitors, glucagon-like peptide 1 receptor agonists). They conclude that insulin secretagogue agents need caution because of their significant hypoglycemic risk. When used, short-acting sulfonylureas (e.g., gliclazide) or glinides (e.g., repaglinide) should be preferred.

Hypothyroidism in the elderly is another debated issue that impacts on many aspects of cognitive impairment and on surgical outcomes. Calsolaro et al. summarize the recommendations for a correct diagnostic workup and therapeutic approach to older people with increased TSH values, especially with regard to the presence of frailty, comorbidities, and poly-therapy. Vacante et al. report evidence coming from few randomized clinical trials investigating the association between non-thyroidal illness (or low-T3 syndrome) and adverse surgical outcomes. They recommend to postpone elective surgery in elderly patients with hypothyroidism until the euthyroid state is achieved. If patients need urgent or emergent surgery, it is recommended to proceed with surgery only in case of mild or moderate hypothyroidism. In this setting, a strong association between T3/T4 ratio reduction recently emerged as surrogate index of frailty and independent marker of survival (1).

Adrenal function in the elderly is a controversial issue for clinicians. In the presence of chronically increased glucocorticoid levels, normal stress response in the elderly is impaired, leading to other age-related changes, including loss of muscle mass, hypertension, osteopenia, visceral obesity, and diabetes. Yiallouris et al. discuss on the complexity of the adrenal hormone changes observed throughout the normal aging process, including surgical procedures. In contrast to the increase in glucocorticoid levels, other adrenocortical hormones, particularly serum aldosterone and DHEA, show significant decreases in the elderly. Gorini et al. provide robust evidence that aldosterone and the mineralocorticoid receptor (MR) dysregulation may play a relevant role in the control of cardiovascular and metabolic functions in the elderly by promoting vasoconstriction and acting as potent pro-fibrotic agents in cardiovascular remodeling. Also, MR contributes to increase blood pressure with aging by regulating myogenic tone, vasoconstriction, and vascular oxidative stress. In addition, dysregulation of MR signaling is associated with hypertension, obesity and diabetes, representing an important cause of increased cardiovascular risk. Plasma aldosterone concentrations decrease in the elderly as well as skeletal muscle content. Interestingly, in a human model of aldosterone excess [primary hyperaldosteronism (PA)], Kwak et al. demonstrate that skeletal muscle mass of women with PA was lower than controls, suggesting that excess of aldosterone may exert adverse effects on skeletal muscle metabolism. The clinical use of MR antagonists is limited by the adverse effects induced by MR blockade in the kidney, rising the risk of hyperkalemia in older patients with reduced renal function.

Musculoskeletal aging is a major public health concern due to high risk of falls, loss of autonomy, and institutionalization with small health outcomes. Bone mineral content is closely related with muscle mass. Several evidence suggest that osteoporosis and sarcopenia share common pathophysiological factors. Furthermore, the correlation between low bone mineral density and sarcopenia in both men and women has been showed. Accordingly, sarcopenia and osteoporosis, which are typical features of aging, are often associated with each other and with the frailty syndrome. Greco et al. investigated the interplay between frailty syndrome, typical of the older people, and the reduction in the quality of life and mobility. By contrast, Vitale et al. discussed the potential role of the IGF-1 system in the modulation of longevity, hypothesizing that the endocrine and metabolic adaptation observed in centenarians and in mammals during caloric restriction may be a physiological strategy for extending lifespan through a slower cell growth/metabolism, a better physiologic reserve capacity, a shift of cellular metabolism from cell proliferation to repair activities and a decrease in accumulation of senescent cells. In line with this clinical evidence, c-Kit, a type III tyrosine kinase receptor, is involved in multiple intracellular signaling whereby it is mainly considered a stem cell factor receptor, participating in vital functions of the mammalian body, including the human. Marino et al. found that c-kit haploinsufficiency in c-kit-deficient mice causes a worsening of myocardial repair after injury and accelerates cardiac aging, thus suggesting that the adult myocardium relies on c-kit expression to regenerate after injury and to counteract aging effects on cardiac structure and function.

Sexual and reproductive functions should be considered as complimentary issues for healthy aging. It is known that older people are interested or still engaged in sexual activities independently of gender. The age-related decline of testosterone often determines unresponsiveness to erectogenic drugs. Meta-analytic data addressed to phosphodiesterase type-5 inhibitors (PDE5i) a protective role on the cardiovascular health in patients with decreased left ventricular ejection fraction so that the addition of testosterone to a PDE5i may represent a successful strategy to prevent male sexual dysfunctions in the presence of reduced testosterone levels, as suggested by Aversa et al. By contrast, psychosocial factors play a critical role in sexual functioning of elderly women, but the anatomical and hormonal integrity of the urogenital system importantly affects many aspects of postmenopausal women's health as well, including the sexual function. A proper assessment of this system should encompass genital symptoms (dryness, burning, itching, irritation, bleeding), sexual symptoms (dyspareunia and other sexual dysfunctions) and urinary symptoms (dysuria, frequency, urgency, recurrent urinary infections). Nappi et al. recommends to fully evaluate all these aspects to enhance physical, emotional and mental well-being in elderly post-menopausal women desiring sexual life. Finally, Gallo et al. provide evidence addressing to advanced age a negative role for successful reproduction also in the male gender, by reporting that in their Assisted Reproduction Center, male age >43 years-old doubles the probability of obtaining poor quality embryos compared to younger men.

The development of neuroimaging has opened new perspectives in clinical and basic research and has modified the concept of brain aging. Tigano et al. suggest that in the near future, neuroimaging will play an increasingly important role in the definition of the individual's brain aging in every phase of the physiological and pathological process and discuss ethical and legal aspects related to precocious diagnosis of brain degenerative diseases with regard to social and clinical implications.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to this Editorial Article, and approved it for publication.

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REVIEW
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ROLE of IGF-1 System in the Modulation of Longevity: Controversies and New Insights From a Centenarians' Perspective

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Human aging is currently defined as a physiological decline of biological functions in the body with a continual adaptation to internal and external damaging. The endocrine system plays a major role in orchestrating cellular interactions, metabolism, growth, and aging. Several *in vivo* studies from worms to mice showed that downregulated activity of the GH/IGF-1/insulin pathway could be beneficial for the extension of human life span, whereas results are contradictory in humans. In the present review, we discuss the potential role of the IGF-1 system in modulation of longevity, hypothesizing that the endocrine and metabolic adaptation observed in centenarians and in mammals during caloric restriction may be a physiological strategy for extending lifespan through a slower cell growing/metabolism, a better physiologic reserve capacity, a shift of cellular metabolism from cell proliferation to repair activities and a decrease in accumulation of senescent cells. Therefore, understanding of the link between IGF-1/insulin system and longevity may have future clinical applications in promoting healthy aging and in Rehabilitation Medicine.

Keywords: IGF-1, insulin, longevity, centenarians, caloric restriction, aging, rehabilitation medicine

INTRODUCTION

Aging is defined as a physiological decline of biological functions in the body with a progressive decline or loss of adaptation to internal and external damaging. In humans the aging phenotype is extremely heterogeneous and can be described as a complex mosaic resulting from the interaction of several stochastic and environmental events, genetic, and epigenetic alterations accumulated throughout the lifetime. Despite its enormous complexity, the molecular basis of aging is limited to few highly evolutionarily conserved biological mechanisms responsible for body maintenance and repair (1).

During the last 3 decades one of the most discussed topics in gerontology is the role of the growth hormone (GH)/insulin-like growth factor-1 (IGF-1)/insulin system in the regulation of longevity. Accumulating evidence suggests that this pathway plays an essential role in the pathogenesis of several age-related diseases including cancer, dementia, cardiovascular, and metabolic diseases (2–4).

In animal models it was shown that down-regulation of the GH/IGF-1/insulin system significantly prolongs the lifespan. However, in humans data are contradictory (5, 6).

This review describes the latest advances in the research of the IGF-1 system and modulation of longevity, hypothesizing that the endocrine and metabolic adaptation observed in centenarians and in mammals during caloric restriction may be a physiological strategy for extending lifespan through a slower cell growing/metabolism, a better control in signal transmission and physiologic reserve capacity and a decrease in accumulation of senescent cells. A review of the literature was conducted using PubMed database with the following keywords: “IGF-1” or “IGF-I” and “longevity.” The search included articles published in the English language between January 2008 and August 2018.

IGF-1 SYSTEM AND LONGEVITY IN ANIMAL MODELS

IGF-1 system has several pleiotropic effects on biological aging (Figure 1). IGF-1 plays a relevant role in fetal development, growth during childhood and adolescence, and adult tissue homeostasis. In addition, IGF-1 seems to have atheroprotective actions, neural protective, and insulin-like effects (at high concentrations) and to regulate skeletal metabolism and muscle regeneration. Nevertheless, IGF-1 is a main risk factor in several tumors due to its potent proliferative activity, mainly through the modulation of cell cycle, apoptosis, and cell survival (7–9). Most of these effects are mediated through the interaction with insulin receptor substrate (IRS)-1 and-2 and the modulation of the PI3K/AKT/ mammalian target of rapamycin (mTOR) pathway (Figure 2).

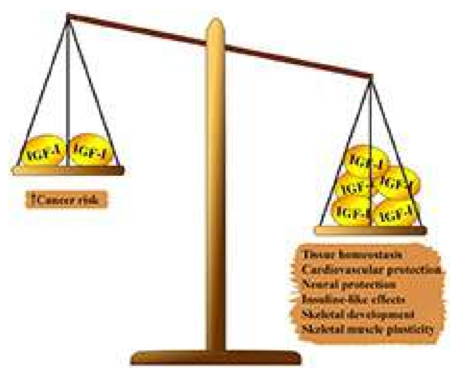

FIGURE 1. Pleiotropic effects of IGF-1 on health status.

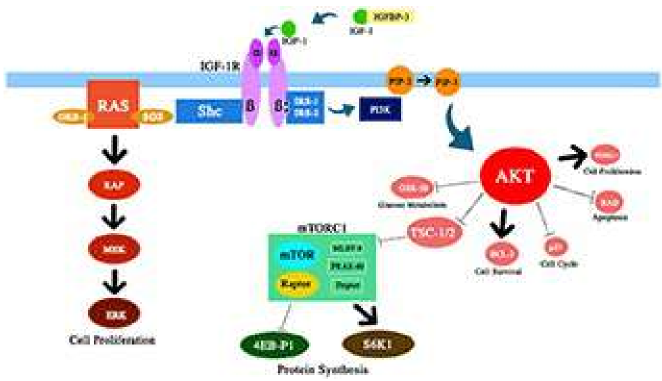

FIGURE 2. Schematic and simplified representation of the several components of the IGF-1/PI3K/AKT/mTOR pathway discussed in this review. IGF-1 increases the activity of AKT protein with relevant effects on cell survival and proliferation, glucose metabolism and protein synthesis.

Several preclinical studies reported that mutation in genes controlling the GH/IGF-1/insulin signaling pathway can significantly increase lifespan in both invertebrate and vertebrate animal models (5, 6).

Invertebrate Models

In invertebrates, the insulin/IGF-like cascade is regulated by several peptides, able to interact with a single, common insulin/IGF-1-like receptor.

In the nematode Caenorhabditis elegans the insulin/IGF-like pathway consists of several proteins encoded by the genes daf-2 (insulin/IGF-1 receptor-like protein), age-1 (encoding the catalytic subunit of PI3K), akt-1, akt-2, pdk-1, sgk-1 (serine-threonine kinases), daf-16 (forkhead transcription factor and the major target of insulin-like signaling in Caenorhabditis elegans), skn-1 (oxidative-stress-responsive transcription factor) and daf-18 (PTEN, a phosphatase, involved in inhibition of the AKT signaling pathway). The reduced activity of daf-2, age-1, akt-1, akt-2, pdk-1, sgk-1 genes were shown to downregulate this pathway, and the animals with these mutations were reported to age more slowly and to have an increased lifespan up to 300%. In contrast, the stimulation of the insulin/IGF-like pathway decreases the lifespan of nematodes (10, 11).

In the fruit fly Drosophila melanogaster the insulin/IGF-like signaling consists of the dINR (Insulin /IGF-1 receptor-like protein), the insulin receptor substrate CHICO, the PI3K Dp110/p60, and the PI3K target PKB (akt-1). The flies with mutation in these genes were reported to have significantly increased longevity (12, 13).

Surprisingly, the same molecular mechanisms in different tissues do not influence aging equally. Several studies in nematodes and fruit flies have suggested that reduced insulin/IGF-like signaling in nervous and adipose tissues has the major role in regulation of longevity (14, 15). Although in invertebrate models it was shown that this cascade is relevant in the modulation of lifespan, the influence of insulin/IGF-like signaling on longevity is much more complex in vertebrates, since they have functionally specific insulin and IGF molecules, IGF binding proteins (IGFBPs), IGFBP proteases, GH, multiple receptors and several mechanisms of intracellular signaling with different tissue specific expression (16).

Vertebrate Models

Several GH/IGF-1 mutant mice have been developed with different targets. The most relevant models are described below.

Snell and Ames Mice

Snell and Ames mice are two mouse strains with mutations in the PIT-1 and PROP-1 genes, respectively (17, 18). Since both PIT-1 and PROP-1 proteins are required for the differentiation of pituitary cells that produce GH, prolactin and TSH, both types of homozygous mutant mice lack all three hormones (18). These models have shown remarkable extension of longevity (42–70% more than wild type mice), enhanced insulin sensitivity and lower tumor incidence (19, 20). When Ames dwarfs were exposed to caloric restriction, their lifespan increased even further (21). Although these animals lack three hormones, it has been demonstrated that lifespan extension is mainly influenced by the GH deficiency (22).

Lit/lit Mice

Lit/lit mice are GH-deficient, carrying a mutation in the gene which encodes the GH-releasing hormone receptor (GHRHR). These animals were dwarfs, showed increased adiposity, lower tumor incidence and a lifespan increased by 23–25% (19).

GH-Releasing Hormone-Knockout (GHRH-KO) Mice

GH-releasing hormone-knockout (GHRH-KO) mice live 43% (in females) and 51% (in males) longer than wild-type animals and share many phenotypic characteristics with Ames dwarf mice, such as enhanced insulin sensitivity, reduction in plasma triglyceride and cholesterol levels, increase in adiposity, plasma leptin, and adiponectin levels (23).

The GH-Receptor-Knockout (GHR-KO) Mice

The GH-receptor-knockout (GHR-KO) mice has elevated serum GH levels and very low IGF-1 levels. Also this strain of mice was reported to live 38–55% longer than wild-type (24) and showed attenuation in oxidative stress, as well as a lower and delayed onset of fatal tumors (25). Similar results were observed in df/KO mice, crossing GHR-KO mice and Ames dwarfs, that lacked both GH and GH receptor and maintained extended longevity (26). Unlike wild siblings and Ames dwarf mice, caloric restriction did not further enhance longevity of GHR-KO mice, suggesting that the GH/IGF-1 axis and caloric restriction might have similar or partly overlapping mechanisms for lifespan prolongation (27).

GH Receptor Antagonism (GHA)

Not all animal models with suppression of GH/IGF-1 system exhibit an increase in lifespan. The GHA mouse strain is one such example. GHA, generated by the substitution of one amino acid (Gly199 Arg in bovine GH), is able to bind the GH receptor with the same affinity as GH, but does not cause intracellular signaling. The lifespan of GHA mice was not significantly increased (28).

IGF-1R$^{+/-}$ Mice

While most of the IGF-1 receptor null mice (IGF-1R$^{-/-}$) die at birth, the animals heterozygous for a mutated allele of the IGF-1 receptor (IGF-1R$^{+/-}$) showed very low serum IGF-1 levels, about 10% smaller size and a 33% increased lifespan in females and 16% in males. However, in this study the wild-type controls lived to only 19 months of age, compromising the interpretation of results (29). More recent studies evaluating the lifespan in another IGF-1R$^{+/-}$ line exhibited a mild 5–10% increase in lifespan, but only in females (30, 31). In addition, the underlying background strain seems to influence the degree of life extension in several murine models (32).

A Brain-Specific IGF1-R$^{+/-}$

A brain-specific IGF1-R$^{+/-}$ mutant lived 9% longer than wild-type, underling the relevant role of the neural system in the modulation of longevity (33).

Liver-Specific IGF-1-Disrupted Mice (LI-IGF-1$^{-/-}$ Mice)

Liver-specific IGF-1-disrupted mice (LI-IGF-1$^{-/-}$ mice) have very low serum

IGF-1 levels and high serum GH levels due to inactivation of the IGF-1 gene. LI-IGF-1$^{-/-}$ mice exhibited markedly decreased adiposity and as a result had 25% lower weight than wild-type mice. Only female LI-IGF-1$^{-/-}$ mice showed a 16% increase in lifespan compared to that observed in control mice (34).

Pappa$^{-/-}$ Mice

Pappa$^{-/-}$ mice are the knockout for the pregnancy associated plasma A (PAPPA) gene, a specific protease for IGF binding proteins. The mean lifespan of this mouse strain was 38% longer compared to wild type controls. Pappa$^{-/-}$ mice were dwarfs, but their serum glucose, insulin, IGF-1 and GH levels were not different from those of wild-type controls, suggesting that PAPPA acts mostly at autocrine or paracrine level and providing evidence for the role of local availability of IGF-1 in the modulation of longevity. In addition to extended longevity, Pappa$^{-/-}$ mice showed a lower incidence of tumor development, as well as age related degenerative lesions (35, 36).

IRS Disrupted Mice

IRS-1 and -2 are important mediators for insulin, as well as for IGF-1 signaling. IRS1$^{-/-}$ mice were insulin-resistant, with a defect in insulin signaling mainly in muscle tissue, about 30% smaller in size than the wild-type and only in females the lifespan was 18% longer compared with wild-type animals (37). IRS2$^{-/-}$ mice were also insulin-resistant, but unlike IRS1$^{-/-}$ mice, they exhibited defects in insulin signaling in more tissues, including the liver, the adipose tissues, and skeletal muscles. These mice developed diabetes, and had a much shorter lifespan than wild-type and IRS2$^{+/-}$ mice. IRS2$^{+/-}$ mice had improved insulin sensitivity and an increased lifespan (+18%) compared to wild-type mice. In addition, brain specific IRS2$^{+/-}$ and IRS2$^{-/-}$ mice were reported to be insulin resistant, and lived 18 and 14% longer than wild-type controls, respectively (38).

KLOTHO Modified Mice

Protein KLOTHO inhibits insulin and IGF-1 signaling, possibly by disrupting receptor/ligand interaction. Mice overexpressing KLOTHO were reported to have normal size, and males developed insulin resistance, while lifespan in both males and females was significantly increased (+18 and +30%, respectively) (39, 40).

P66shc Disrupted Mice (P66shc$^{-/-}$ Mice)

P66shc is a protein mediating IGF-1 post-receptor signaling by activating the

MAPK pathway. P66shc$^{-/-}$ mice had normal phenotype, but lived 28% longer than wild-type controls (41). However, these data were not confirmed in a recent study (42).

The role of GH/IGF-1/insulin signaling in aging and longevity has been deeply studied through all these animal models. While in invertebrates the impact of downregulation in the IGF-1/insulin pathway on lifespan resulted to be clear and considerable, in murine models this effect was attenuated and not reproducible in some cases, such as in the IGF-1R$^{+/-}$ and P66shc$^{-/-}$ mice. However, most of these models showed the presence of some commonalities among the long-lived mice, such as reduced circulating IGF-1 and insulin levels and increased insulin sensitivity, which likely contribute to reduce tumor incidence, to improve stress resistance and to extend the lifespan. Genetic alterations able to disrupt IGF-1 system can keep the animals healthier for longer periods and can postpone or alleviate some age-related diseases. In this process nervous and adipose tissues seem to have a relevant role.

Additionally, more data are needed to determine the best time point during the lifetime for intervention in suppressing IGF-1 system to obtain beneficial effects on lifespan. In igf$^{f/f}$ *C57B*l/6 mice deficiency in circulating IGF-1, starting at 5 months of age or earlier, increased lifespan by 15% only in females, with a reduction in the number of organs exhibiting disease pathology at the end of life compared to control group. Moreover, late-life IGF-1 deficiency (15 months) reduced cancer risk but had no beneficial effects on lifespan (43). These data underline the importance of IGF-1 deficiency when started early in life for increasing longevity. On the other hand, Mao et al. (44) recently reported that late treatment of 18-months old CB6F1 mice with an anti-IGF-1 receptor monoclonal antibody prolonged lifespan by 9% in females and improved several aspects of healthspan.

IGF-1 SYSTEM IN LONG-LIVED INDIVIDUALS

Centenarians are considered the best human model to study biological determinants of longevity having reached the very extremes of the human lifespan (45).

Several studies compared circulating insulin and IGF-1 levels in centenarians with those of younger controls (46).

Metabolic age-dependent remodeling is a physiological process occurring in the whole population. Aging is frequently associated with a decline in glucose tolerance secondary to an increased insulin resistance (47), but an exception occurs in long-lived people. Paolisso et al. (48) found that insulin resistance increased with aging and declined in subjects older than 90 years living in Southern Italy. Indeed, long-lived subjects showed a higher insulin sensitivity and a better preservation of beta-cell function than younger subjects. Such difference was also independent of the main anthropometric and metabolic confounders. Centenarians had a lower 2-h plasma glucose concentration than that aged subjects (mean age 78 years) during oral glucose tolerance test. In centenarians insulin-mediated glucose uptake was greater than in aged controls during euglycemic glucose clamp, supporting a preserved glucose tolerance and insulin action in this long-lived group (49, 50). Similar results, supporting a better insulin sensitivity, were observed in other long-lived populations (51, 52).

Furthermore, centenarians showed a preserved insulin action not only on the glucose metabolism but also on adipose tissue. In fact, insulin infusion is normally associated with inhibition of lipolysis and thus to a significant decline in plasma free fatty acid and triglyceride concentrations. In centenarians the inhibitory activity of insulin on lipolysis was stronger than that of controls (mean age 78 years) (50). It is noteworthy that centenarians compared to aged controls have also a lower sympathetic tone which might be due to a better insulin action and thus, to a low fasting plasma insulin levels (53, 54).

Data on IGF-1 system in relation to longevity are still controversial in long-lived subjects (46). Paolisso et al. (55) described an increased plasma IGF-1/IGFBP-3 ratio in healthy centenarians compared to elderly subjects. They hypothesized that this elevated ratio was indicative of a higher IGF-1 bioavailability which contributed to the improved insulin action in centenarians. In contrast, Bonafè et al. (56) reported that subjects with at least an A allele of the IGF-1 receptor gene (G/A, codon 1013) had low levels of free plasma IGF-1 and were more represented among long-lived people. Arai et al. (57) described relatively low levels of serum IGF-1 in a population of Japanese centenarians. In this population the lowest tertiles of both IGF-1 and IGFBP-3 were associated with increased mortality (58).

These conflicting results probably reflect the complexity of the IGF-system and ethnic differences in enrolled populations. In addition, centenarians have often been compared to a control group of younger subjects. Therefore, in most of these studies it was not possible to conclude if IGF-1 differences between both groups were related to a different lifespan or reflected a physiological age-dependent IGF-1 decline. Indeed, there are several limitations to study centenarians: (1) low prevalence (1 centenarian per 5–10.000 inhabitants), (2) presence of frailty due to extreme age (almost 95% of centenarians have at least

1 frailty criterion), (3) lack of a control group of the same age (45, 59). Due to these limitations, this human model is unsuitable to study age-dependent variables that may be involved in the modulation of the lifespan.

Centenarians' offspring represent another interesting model to define relevant factors involved in human longevity and healthy aging. A concordant set of observations in different countries suggest that centenarian's offspring are healthier than members of the same demographic cohorts (51, 60, 61) and biologically (epigenetically) younger than their chronological age (62). Overall, these studies indicate that relatives of centenarians have a high probability for living longer and in good health (60, 63). In addition, studying centenarians' offspring has the relevant advantage of the availability of an appropriate demographically matched control group, consisting in age-matched offspring having both parents born in the same birth cohort of centenarians, but dead before the threshold age over which subjects were classified “long-lived.” This strategy is crucial for avoiding cohort effects. Therefore, centenarians' offspring model can overcome some limitations that are found in the study of centenarians (rarity, frailty and lack of an appropriate control) (60).

In few studies the IGF-1/insulin system has been characterized in centenarians' offspring and an appropriate matched control group.

We have evaluated circulating IGF-1 bioactivity, measured by an innovative IGF-1 Kinase Receptor Activation (KIRA) Assay in centenarians, centenarians' offspring and offspring matched-controls. Centenarians and centenarians' offspring had relatively lower circulating IGF-1 bioactivity compared to controls. Interestingly IGF-1 bioactivity in centenarians' offspring was inversely associated to insulin sensitivity (51).

Suh et al. (64) evaluated serum IGF-1 levels in Ashkenazi Jewish centenarians' offspring and in age-matched controls. Female centenarians' offspring had 35% higher serum IGF-1 levels than that controls. This difference may represent a compensatory response to reduced IGF-1 receptor signaling. Indeed, female offspring showed shorter stature than controls. In addition, an overrepresentation of heterozygous mutations in the IGF-1 receptor gene together with relatively high serum IGF-1 levels and weakened activity of the IGF-1 receptor has been described in Ashkenazi Jewish centenarians compared to controls without familial longevity.

In order to study longevity, other authors characterized these pathways in nonagenarian siblings and their offspring. In the Leiden Longevity Study, 421 families were recruited consisting of at least two long-lived Caucasian siblings, their offspring and partners of the offspring as control. In these populations serum glucose, insulin and triglycerides were the best biomarker of healthy aging (glucose and insulin low levels were considered healthy) (65). Nonagenarians in the lowest circulating IGF-1/IGFBP-3 ratio were associated with a better survival (66). The offspring of familial nonagenarians exhibited a better insulin sensitivity compared to their partner, while similar non-fasted serum levels of IGF-1 and IGFBP-3 were observed between both groups (67). Interestingly, 24-h total GH secretion was 28% lower in offspring compared with controls (68).

Another approach adopted to study longevity in humans consists in the selection of familial components of exceptional longevity and healthy aging, based on strict criteria, such as the Family Longevity Selection Score adopted in Long Life Family Study. These families enriched for exceptional life expectancy were compared to controls without family history of longevity (69). In this population circulating IGF-1 levels resulted to be a valid age-related biomarker (70).

In support of the potential role of the GH/IGF-1/insulin system in the human longevity, there are many genetic studies. Indeed, several genetic loci have been identified to be associated with circulating IGF-1 and IGFBP-3 levels and potentially able to affect aging (71). A genome-wide association analysis performed in nonagenarians and a population of subjects <60 years of age, showed a clear association between genetic variation of genes involved in insulin/IGF-1 pathway and human longevity (72). In a prospective study of older people, females with a genetic profile suggestive of a decreased insulin/IGF-1 signaling activity, exhibited a longer survival (73). In four independent cohorts of long-lived individuals it has been recently described a linear increased prevalence of GH receptor exon 3 deletion (d3-GHR) homozygosity with age. The presence of d3/d3 genotype increased life expectancy by about 10 years (74).

IGF-1 SYSTEM AND CALORIC RESTRICTION

One of the most robust striking observations in the biology of aging is the capability of caloric restriction to prevent or delay several age-related diseases and to increase lifespan in mammals (75–78). The biological mechanisms of this phenomenon are not completely clear, but it has been suggested a potential involvement of relevant alterations in energy metabolism, endocrine system and oxidative damage.

Caloric restriction instigates numerous hormonal changes. In rodents caloric restriction without malnutrition suppressed circulating IGF-1 and insulin levels in proportion to the level of restriction, increased insulin sensitivity and resistance to stress and toxicity, and reduced the cancer risk (79, 80). Interestingly, most of these characteristics observed in wild type mice during caloric restriction resemble those reported in mice that are long-lived due to genetic disruption of the GH/IGF-1/insulin signaling, as previously described. In humans, randomized clinical trials showed that caloric restriction does not attenuate serum IGF-1 levels unless protein intake is reduced (81, 82). However, a recent meta-analysis, evaluating the effect of dietary restriction on well-recognized biomarkers of healthy aging, showed a decrease in circulating IGF-1 levels in humans (83). In addition, during caloric restriction skeletal muscle transcriptional profile showed a suppression of local insulin/IGF-1 pathway inducing a younger transcription profile (84).

Other circulating hormonal changes, such as decreased insulin, thyroid hormones and leptin levels, and increased adiponectin levels and insulin sensitivity have been observed during dietary restriction (85, 86). This hormonal adaptation may have a relevant role in extension of lifespan through several mechanisms:

1) *Reducing metabolic rate, cell proliferation, and oxidative stress*. In fact, IGF-1 is a potent growth factor and thyroid hormone is a potent stimulator of basal metabolic rate and oxidative metabolism. In addition, transcriptional patterns suggest that chronic moderate caloric restriction in adult individuals retards the aging process by shifting cellular metabolism from growth to maintenance and repair activities (84).
2) *Decreasing the accumulation of senescent cells*. Cellular senescence has been demonstrated to be a key mediator of aging (87). Over time protein homeostasis declines and damage accumulates. Interestingly, it is possible to delay several age-related diseases through attenuating the accumulation of senescent cells (88, 89). Normally the mTOR pathway is activated by several signals, including nutrients, IGF-1 and insulin (Figure 2). The down-regulation of this pathway, reported after caloric restriction, increased lifespan in several organisms. This effect seems to be secondary to an up-regulation of autophagy, a cytoprotective self-digestive process. In fact, autophagy is a cellular recycling process that can remove aged or damaged cellular components preventing the accumulation of senescent cells (90, 91).
3) *Counteracting inflammaging*. In both animals and humans dietary intervention can delay the aging process by attenuating low-grade inflammatory status (83, 92). The mechanisms underlying the anti-

inflammatory activity of dietary restriction are not well-defined. It has been hypothesized that this effect is due to the reduction in fat mass and pro-inflammatory adipokines, and to an improvement of intestinal barrier integrity observed during dietary intervention (93, 94).

Interestingly, the endocrine biochemical profile observed in subjects during caloric restriction is comparable to that reported in centenarians, supporting a potential role of the endocrine system in the modulation of lifespan. In addition to an increase in insulin sensitivity and a decrease in plasma/serum IGF-1 levels, several studies showed an increase in circulating adiponectin levels and a reduction in circulating leptin and thyroid hormones levels in long-lived people compared to younger subjects (Table 1).

Table 1. Endocrine biochemical profile observed after caloric restriction and in centenarians compared to younger subjects.

Endocrine parameters	Caloric restriction	Centenarians
IGF-1	=/↓*	↓
Insulin	↓	↓
Insulin sensitivity	↑	↑
Adiponectin	↑	↑
Leptin	↓	↓
Triiodothyronine (T3)	↓	↓

*↓, decrease; ↑, increase; =, no change; *more evident in murine models.*

Adipose tissue is an endocrine organ producing several cytokines involved in relevant processes, such as the energy metabolism, lipid, and glucose homeostasis and modulation of inflammatory response. Visceral adipose tissue has a main role in the development of metabolic diseases (95). Aging is associated with an increase in fat mass and a redistribution of adipose tissue, characterized by loss of peripheral subcutaneous fat and accumulation of visceral fat. In elderly, alterations in the secretion, synthesis and function of the adipokines have been described, probably due to an unbalance in the function, proliferation, size, and number of adipose cells (86). Adiponectin is an insulin sensitizing, anti-inflammatory and anti-atherogenic cytokine. Adiponectin circulates in the blood in several forms: trimer, hexamer, high molecular weight (HMW) multimer, and globular adiponectin (a proteolytically cleaved form).

The HMW multimer is believed to be the more active form of adiponectin at protecting against insulin resistance and diabetes (96). Circulating adiponectin is independently and negatively related to facets of the metabolic syndrome, including insulin resistance, body weight, blood pressure, and serum lipids. Leptin is mainly produced in the subcutaneous and to a lesser extent in the visceral white adipose tissue. This cytokine regulates food intake, energy expenditure and atherogenesis. Leptin boosts weight loss by reducing appetite and stimulating metabolic rate and has pro-inflammatory properties (97).

Several studies reported that centenarians have higher plasma adiponectin and lower leptin concentrations than younger controls (53, 98–102). All forms of adiponectin were significantly increased in centenarians, but the HMW multimer was markedly higher (99). In centenarians the high adiponectin concentrations resulted to be independent of BMI, renal or cardiovascular function and were associated with a favorable metabolic phenotype (higher HDL-C, lower hemoglobin A1c, insulin, HOMA-IR and triglycerides) (98, 99). Increased adiponectin levels were also detected in the offspring of the long-lived subjects (older than 95 years) (103).

A decrease in thyroid hormones levels seems to be peculiar in centenarians. Mariotti et al. (104) reported that healthy centenarians had lower serum TSH and FT3 levels and higher serum rT3 levels compared with that observed in other control groups. In another Italian population of centenarians total T4 values were lower than normal range in 60% of examined subjects (105). Baranowska et al. reported that serum T3 levels in centenarians were lower compared with that observed in early elderly and young women (52). We have recently characterized thyroid function profile in an Italian cohort of 672 subjects (range 52–113 years old). An age-dependent decrease in FT3 level and FT3/FT4 ratio has been observed, while FT4 and TSH increased with aging (106). In Chinese centenarians' families a decline in thyroid function (high TSH and low FT3 concentrations) appears to be associated with age, and this phenotype is heritable (107). Corsonello et al. (108) found in relatives of centenarians (offspring or nieces/nephews) lower comorbidities, FT3, FT4, and TSH levels than age-matched controls who were not relatives of centenarians. In another Italian population lower plasma level of FT4 were observed in centenarians' offspring compared to age-matched controls (60).

In general, centenarians are lean (109) and follow healthy nutritional habits but without a calorie-restricted diet (110). Similarly to subjects during caloric restriction, a slower cell growing/metabolism, a better control in signal transmission and an enhanced autophagy have been observed in centenarians.

Through a genome-wide DNA methylation analysis in centenarians and their offspring, we have identified epigenetically modulated genes and pathways potentially involved in the process of aging and longevity. Our results suggest that a better preservation of DNA methylation status, a slower cell growing/metabolism and a better control in signal transmission through epigenetic mechanisms characterized these populations (111). Centenarians have a preserved bioenergetic function through a mitochondrial hypertrophy that can recompense for functional defects (112). In addition, healthy centenarians have high levels of autophagy, as indicated by higher serum beclin-1 levels compared with both young patients with myocardial infarction and healthy controls (113). An increase in autophagic activity has been also observed in subjects belonging to families with exceptional longevity (114).

A relevant divergence occurs concerning the inflammatory status, which is attenuated in subjects after caloric restriction (115, 116) and high in centenarians (117–119). With aging a state of low-grade and chronic inflammatory condition (called inflammaging) and an increased prevalence of several diseases have been observed, such as cardiovascular disease, atherosclerosis, tumors, cognitive impairment, osteoarthritis, and diabetes (120, 121). Therefore, attenuation of chronic inflammatory status after caloric restriction represents a beneficial effect. Centenarians show signs of inflammaging but at the same time seem to be spared from its deleterious consequences. This apparent paradox can be explained by the fact that centenarians possess a complex and peculiar balancing between pro-inflammatory and anti-inflammatory factors, resulting in a slower, more limited and balanced development of inflammaging, in comparison with elderly, who are characterized by an inappropriate response to counteract chronic inflammation (120, 121).

These findings suggests common mechanisms to increase lifespan and to delay age-related diseases adopted in centenarians and in mammals following a calorie-restricted diet.

AUTHOR'S OPINION

Preclinical models have provided a great insight into the aging process with consistent data considering the role of the GH/IGF-1/insulin system in the modulation of lifespan. While it is well known that enhanced insulin sensitivity and low insulin levels are associated with an improved survival, there are several evidences showing that attenuation of the GH/IGF-1 axis may have beneficial effects in extending lifespan in humans. However, it is still unknown which are the optimal IGF-1 levels during life to live longer and healthier. In addition, IGF-1 receptor sensitivity and activation of the post-receptor pathway were not evaluated in the majority of the study enrolling long-lived subjects. Therefore, it is not possible to define the real activation status of the IGF-1 receptor signaling through the mere dosage of circulating IGF-1 levels. This renders more difficult the identification of pharmacological or environmental strategies targeting this system for extending lifespan and promoting healthy aging. A comprehensive understanding of these aspects remains a major challenge for uncovering interventions to slow human aging and to adopt in Rehabilitation Medicine. Future studies should evaluate the functional status of IGF-1 receptor signaling, also through transcriptional profiling and functional network analyses concerning IGF-1 regulated genes, in long-lived subjects.

CONCLUSIONS

Striking similarities have been described concerning endocrine profile between centenarians and subjects after a calorie-restricted diet. The endocrine and metabolic adaptation observed in both models may be a physiological strategy to increase life span through a slower cell growing/metabolism, a slower loss of physiologic reserve capacity, a shift of cellular metabolism from cell proliferation to repair activities and a decrease in accumulation of senescent cells. These mechanisms seem to be, at least in part, mediated through the modulation of the GH/IGF-1/insulin system.

AUTHOR CONTRIBUTIONS

GP and MV researched all the data from available scientific literature on the PUBMED database. GV interpreted all data, organized, wrote, and revised the whole manuscript, and also conceptualized and drew all the figures assembling the final formatted review. LH organized and revised the whole manuscript.

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REVIEW
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Adrenal Aging and Its Implications on Stress Responsiveness in Humans

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Normal aging results in subtle changes both in ACTH and cortisol secretion.

Most notable is the general increase in mean daily serum cortisol levels in the elderly, without a noteworthy alteration in the normal circadian rhythm pattern. Glucocorticoid excess seen in the elderly population can have serious consequences in both the structural and functional integrity of various key areas in the brain, including the hippocampus, amygdala, prefrontal cortex, with consequent impairment in normal memory, cognitive function, and sleep cycles. The chronically elevated glucocorticoid levels also impinge on the normal stress response in the elderly, leading to an impaired ability to recover from stressful stimuli. In addition to the effects on the brain, glucocorticoid excess is associated with other age-related changes, including loss of muscle mass, hypertension, osteopenia, visceral obesity, and diabetes, among others. In contrast to the increase in glucocorticoid levels, other adrenocortical hormones, particularly serum aldosterone and DHEA (the precursor to androgens and estrogens) show significant decreases in the elderly. The underlying mechanisms for their decrease remain unclear. While the adrenomedullary hormone, norephinephrine, shows an increase in plasma levels, associated with a decrease in clearance, no notable changes observed in plasma epinephrine levels in the elderly. The multiplicity and complexity of the adrenal hormone changes observed throughout the normal aging process, suggests that age-related alterations in cellular growth, differentiation, and senescence specific to the adrenal gland must also be considered.

Keywords: senescence, adrenal cortex, stress, HPA axis, glucocorticoids

INTRODUCTION

Normal aging is associated with multi endocrine changes, including those associated with changes in the structure and function of the adrenal gland. The various morphological changes of the adrenal gland that occur during aging are associated with alterations in hormonal output, such as a gradual sustained, increase in glucocorticoid secretion and decline in adrenal androgen levels. The increase in circulating levels of cortisol in aging individuals is of particular interest due to the impact of cortisol on several systems, including cognition, and the inherent relationship of chronic stress, elevated cortisol, and aging.

Stress is a constant factor in modern life. The stress response in healthy organisms is aimed at maintaining the balance of biological functions, or homeostasis, when faced with physiological or psychological challenges, that may be real or even perceived. The normal stress response entails a tight orchestration of several adaptive response cascades of the central nervous system and the neuroendocrine systems that are targeted at facilitating homeostasis and ultimately, survival. An integral part of the response entails activation of stress neural circuits, which link brain regions responsible for basic sensory and motor functions for perception and motor response to the stressful challenge, respectively, as well as more intricate autonomic, neuroendocrine, cognitive, and behavioral activities. While activation of these neural circuits is considered part of the normal stress response, chronic stress may deregulate these circuits and responses, resulting in impaired function of these systems.

The stress response system is comprised of central and peripheral components. Of these, the hypothalmic-pituitary-adrenal (HPA) axis has been defined as a primary player in the stress response. The HPA axis has been the subject of intense basic and clinical research in the attempt to understand why the primary adrenal hormonal output, glucocorticoids, is critical for life. While the stress system has been widely studied, the magnitude, and complexity of the various interactions between the its primary components remain elusive (1). Nerve cells in the lateral paraventricular nucleus (PVN), which secrete corticotropin-releasing hormone (CRH) project toward the hindbrain to regions responsible for arousal and sympathetic function. In return, the PVN receives catecholaminergic fibers through an ascending noradrenergic bundle from the locus ceruleus and central sympathetic system. Upon activation, CRH is released into the hypophyseal portal system, which serves as a conduit between the PVN and the CRH neurons with the pituitary, subsequently stimulating adrenocorticotropic hormone (ACTH), and endorphin release by the pro-opiomelanocortin (POMC) neurons of the arcuate nucleus. While the release of CRH and the subsequent stimulation of brainstem arousal and sympathetic centers is part of a positive, reverberating feedback loop, the release of endorphins and ACTH is part of a negative feedback loop that exert inhibitory effects on CRH secretion. ACTH release into the bloodstream acts on the adrenal cortex resulting in the release of cortisol. Cortisol, in turn, exerts negative feedback, both at the level of the pituitary and the hypothalamus (1). Both the acute and chronic activation of the components of the stress system and HPA axis is associated with direct consequences on the activity and functional integrity and of other physiological systems, including those responsible for reproduction, growth, and immunity, which are mostly attributed to the interaction of adrenal hormones with other physiologic systems (1).

The wealth of the available evidence strongly suggests that chronic stress can accelerate aging (2). In addition, however, there is general support that the ability to terminate the stress response systems in the elderly population is impaired (3). It is not clear whether structural and functional alterations in the aging brain, with a commitment decrease glucocorticoid-mediated feedback inhibition contribute to the cortisol hypersecretion observed in the elderly, or whether this is related to functional changes within the adrenal gland itself. The aim of this review is to address adrenal aging with particular focus on alterations in adrenal cortisol production and its implications on stress responsiveness in the elderly.

Adrenals, Aging, and Stress

Aging and Stress

Aging or senescence has served as a focus for research for several decades. While life expectancy has increased significantly, with the age group consisting of individuals over the age of 85 years being the fastest growing age group, our understanding of the aging process remains unknown. Upon critical examination of numerous theories proposed to explain the aging process, two categories emerge that are not mutually exclusive: (1) those that are based on the notion that aging is programmed; and (2) those that are based on the idea that aging is related to the accumulation of damage at a wide gamma of targets and from various sources (4). The cellular senescence/telomere theory supports the idea of a biological clock and suggests that there is a limited replicative life span of normal cells (5). Cell senescence may be triggered in response to stress through different mechanisms, including mutations in signaling, DNA damage from free radicals, or replication (6). Replicative senescence comes from the spoilage of telomeres, resulting after each cell division, n and can be reversed via activation of telomerase, an enzyme that helps regenerate telomeres (7). In stress-induced senescence, the hypothesis is that DNA undergoes alterations due to extrinsic stressors and intrinsic processes, via mutations on the repair enzymes as a result of the dys-functioning and further aging (7, 8). The gene regulation theory of aging supports the notion that genes are responsible for life and death (9). This theory has been supported by findings showing that some genes are responsible for longevity by decreasing insulin-like signaling, and that the life-span could be regulated, in part, by gene expression, similarly to sirtuin, a family of anti-aging genes (9). Other theories, such as the immunological theory, supports the idea that there is deterioration of the normal function of the immune system across aging, with subsequent increased vulnerability to infectious diseases and death (10, 11). The stress theory of aging, sometimes referred to as hormonal theory, supports the notion that the cumulative effects of stress and stressful environments causes disrupts normal cellular function, cause cellular damage, which eventually is expressed in system dysfunction and aging (12). The frequency of stress-related conditions and diseases, such as anxiety disorders, insulin resistance, hypertension, coronary heart disease, depression, cerebrovascular disease, and others, radically increase throughout the lifespan. Additionally, individual differences in vulnerability and resistance to stress and stress-related pathologies may be attributed in part to the heterogeneity of the aging process (13). Primary signaling pathways that respond to stress include the insulin/IGF, TOR, and sirtuin networks (14). Changes in the nutrient grade or the number of stress stimuli result in alterations in these signaling pathways, which alter their mitochondrial function and metabolic activity, via genome proteostasis and maintenance circuits. The network integration and activity of both the stress response system, as well as the maintenance circuitry, which are aimed to augment endurance, develop during the early developmental period. The available evidence suggests that decreased responsiveness and integration of the various components of the stress response, can contribute to both aging and age-related diseases. An important insight in current aging research is that decreased function throughout aging may not be permanent. Rather, it appears that age-related decline can be stunted and the lifespan increased, by increasing the resistance to stress-related processes via conserved signaling pathways (15).

Adrenal Glands: Structure and Function

The adrenal gland or suprarenal gland weighs about 5 g consists of two distinct structures, both anatomically and chemically: an inner region, or medulla, that contains catecholamine-producing chromaffin cells and an outer region, or cortex, that is important for synthesizing life-sustaining steroids. The medulla, which produces catecholamines receives sympathetic innervation, while the cortex, which produces life-sustaining steroids is regulated by the pituitary hormones (16).

The cortex is divided into three zones; zona reticularis (amounting up to 7% of the gland mass), zona glomerulosa (15%), and zona fasciculata (50%), where each of the zones secrete different hormones (Table 1) (Figure 1). All adrenocortical cells contain excessive quantities of lipids, mainly in the outer part of the zona fasciculata. The two inner zones (zona fasciculata and zona reticularis) produce cortisol and sex hormones, including dehydroepiandrosterone (DHEA). Cortisol and its derivatives are known as glucocorticoids due to their function to stimulate gluconeogenesis, raising blood pressure, and regulate inflammation. Due to its latter property, it is often given to patients with systematic inflammatory conditions (e,g., autoimmune disorders), as well as to transplant patients. The outer cortical zone, zona glomerulosa, produces aldosterone in response to the renin-angiotensin system, which regulates body water, and salt. All zones secrete corticosterone, but the actual mechanisms forming cortisol and sex-related hormones are found in the two inner zones, whereas zona glomerulosa has limited aldosterone synthesis (24).

Table 1. Hormones of the adrenal glands.

Adrenal gland	Associated hormones	Chemical class	Main effect
Cortex: zona glomerulosa	Aldosterone	Mineralocorticoid	Balance water and salt (17)
Cortex: zona fasciculata	Cortisol	Glucocorticoids	Biomolecules (fats, proteins, and carbohydrates) convention to energy (18)
Cortex: zona fasciculata	Corticosterone	Glucocorticoids	Regulate immune response and suppress inflammatory reactions (19)
Cortex: zona reticularis	Androstenolone	Mineralocorticoid	Precursor to male and female sex hormones, testosterone, and estrogen (20)
Adrenal medulla (small amount)	Dopamine	Catecholamines	Regulates pumping strength of the heart and improves blood flow (21)
Medulla: Chromaffin Cells	Adrenaline	Catecholamines	Responds to stress by increasing heart rate (22)
Medulla: Chromaffin cells	Nor-adrenaline	Catecholamines	Vasoconstriction results in high blood pressure (23)

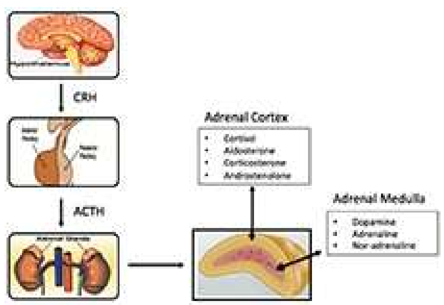

FIGURE 1. Schematic of components of the primary adrenal axis and main hormones produced in adrenal cortex and adrenal medulla.

The centrally-located medulla, which constitutes 28% of the gland, is surrounded by the adrenal cortex, and made up of interlacing cords of densely innervated granule-containing cells adjacent to venous sinuses. The cells comprizing the medulla are derived from the nervous system and produce catecholamines (adrenaline, noradrenaline, and dopamine). Stimulation of hormone secretion, leads to release of the hormones into the circulation via exocytosis (25). The medulla of the adrenal gland is considered an important component of the sympathetic nervous system, and houses two primary cell types, the adrenalin-secreting type [90% of cells], and the noradrenaline-secreting type (10%), (13, 16) along with small numbers of sympathetic ganglion cells. While not essential to life, the medulla significantly helps the organism to cope with stress through adrenalin and noradrenalin secretion, which increase the heart rate, convert glycogen to glucose in the liver, among others (26).

Adrenal Stress Response: HPA Axis Activation

Exposure to a stressful stimulus results in activation of both the hypothalamic-pituitary-adrenal (HPA) axis and the arousal/sympathetic system, which comprise primary components, the central and peripheral parts, of the stress system. Of the variety of factors that are produced and released in the stress response, the mediators of the HPA axis, particularly the glucocorticoids, are critical (1). Normally, after exposure to a stressor, glucocorticoids act on the brain to restore physiological, and behavior homeostasis. Glucocorticoids produce adaptive responses by exerting effects on various central and peripheral sites, in addition to exerting effects on wide span of neuronal activities, such as nerve cell excitability, neuroplasticity, neurogenesis, neuronal death, stress responsiveness, and behavioral responses. The glucocorticoid, namely via cortisol, negative-feedback loop comprises a critical part of the adrenal stress response as it acts to terminate HPA activation. The adrenal steroids appear to exert their effect via the interaction with intracellular receptors that show specific, and high affinity ligand binding. Two types of receptors for adrenal steroids have been identified in the brain and the pituitary (27). Both glucocorticoid receptors have been found in the brain and have been implicated in basal and stress-associated negative feedback control of the HPA axis. The type I, or mineralocorticoid (MR), receptor appears to mediate, and regulate the tonic influences of glucocorticoids on brain functions at basal levels. Activation of the type II, or glucocorticoid (GR), receptor plays an important role in blunting further activity of the stress response through negative feedback suppression of the stress response. Changes in learning and memory, as well as increased anxiety is associated with activation of GR. These functional changes are anatomically encoded within distinct neural regions and structures. The hippocampus (HC) and prefrontal cortex (PFC) are largely inhibitory of the limbic-HPA axis activity, and the amygdala appears to activate the stress response. Elevated levels of glucocorticoids appear to impair synaptic plasticity in the HC and the acquisition of HC-dependent memories. GR and MR are both abundantly expressed in neurons of the HC, PFC, and amygdala. MRs and GRs may have opposing functions in regulating hippocampal synaptic neuroplasticity during the stress-response. Activation of MRs may be a prerequisite for hippocampal plasticity, while GRs may exert an inhibitory effect on plasticity (1, 28–30).

Adrenal Gland Changes With Aging

As physiologic functions gradually decline during aging, a reduction in activity across the hypothalamic-pituitary-adrenal (HPA) axis occurs. The HPA axis is fundamental to homeostasis, acting as a regulator of stress response (31). During the multifactorial process of aging, the secretory pattern of the adrenals, especially of the adrenal cortex, is subject to quantitative and qualitative alterations, and so is the axis's negative feedback sensitivity to the end hormones (32), probably contributing to the pathogenesis of age-related disorders, particularly the decline in cognition observed in older people (33). In the aging population, several studies revealed an improved physical and cognitive performance during higher activity of the HPA axis, compared with reduced activity of the axis (34, 35).

Adrenal Hormone Alterations During Aging

It has been suggested that aging is related to the loss of balance between the two fundamental process, damage, and repair (36), as well as tissue/organ loss over time (37, 38). This natural gradual deterioration of function is modulated by the stress system and weakening of the normal pathways of repair, such as DNA damage repair, mitochondrial metabolism, and proteostasis. In humans, aging is characterized by an increase in adrenal glucocorticoid secretion and a decrease in adrenal androgen synthesis. As aging occurs, several changes in hormone levels taking place.

The cortisol secretion pattern by zona fasciculata of the adrenal cortex undergoes several modifications with age. Unlike most hormones whose levels diminish throughout aging, mean cortisol concentrations increase (39), displaying generally irregular patterns and a flattened circadian profile (40, 41), an evening and night time higher nadir (33, 39), and an attenuated awakening response with an earlier morning level peak (32). Additionally while aging, there is diminished negative feedback on the secretion of cortisol, due to impaired sensitivity of the HPA axis (33, 42). This age-related attenuation of axis negative feedback may be associated with several factors, such as vascular components, reduced number of brain glucocorticoid receptors, differences of cortisol concentration in the cerebrospinal fluid (CSF), and alterations of cortisol clearance in the blood brain barrier or the CSF (42).

Increased cortisol levels and diminished axis sensitivity are generally related with inferior cognitive status, dementia of degenerative and vascular cause (43), depression, and anxiety (39). Furthermore, higher urinary free cortisol concentrations are associated with Alzheimer's disease (44) and increased salivary cortisol concentrations in older people are associated with increased mortality risk, higher risk of diabetes mellitus, and hypertension (45). Additionally, 11-β hydroxysteroid dehydrogenase, which acts to transform cortisone into active cortisol, shows increased activity during aging, affecting tissue cortisol availability (46).

Frailty has also been associated with elevated diurnal cortisol levels (47, 48), a state of increased vulnerability of the aging population. As a catabolic hormone, higher cortisol levels are linked with characteristic clinical features of frailty such as weight loss, muscle mass reduction, and anorexia (49). On the contrary, lower diurnal cortisol levels are associated with longevity (50).

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS), produced and secreted by zona reticularis of the adrenal cortex in response to ACTH stimulation, decrease profoundly during aging (39). Adrenal secretion of DHEA gradually declines over time at a rate of 1 ± 2% per year (42), constituting one of the biggest endocrine changes found in human aging, with a 5- to 10-fold decrease (51) resulting in “adrenopause” (52). By the age of 70–80 years, DHEAS are about 30% of peak values in women and 20% in men, compared with people under 40 years of age (32, 53). In peripheral tissues, DHEA/DHEAS convert into androgens and oestrogens, posing a significant role, especially in older men, where <50% of these androgens are produced from the testicles (32).

DHEA/DHEAS secretion is considered of great significance in frailty (49). Higher levels have been linked with improved health outcomes (51), improved psychological status and functional abilities, muscle strength, higher bone density, anti-inflammatory actions (54), reduced risk of death from cardiovascular disease (55), and increased longevity in males (56). Many cross-sectional studies have found correlation between several diseases (e.g., Alzheimer's disease, type 2 diabetes, and depression) and DHEA-S levels (31). Lower DHEAS levels have been associated with deficient mental health (39), as well as increased cardiovascular mortality and cardiovascular events in people aged over 50 (54).

The reduction in DHEAS levels with the simultaneous preservation of plasma cortisol, reveal a dissociation of the cortical secretory pattern, which may be caused by selective depletion in zona reticularis cells leading to impairment of androgens, rather than being controlled by a hypothalamic aging pacemaker (42, 52). In particular, zona reticularis cells seem to be susceptible to vascular injury and possibly to the intra-adrenal gradient of autocrine and paracrine elements, leading to cell damage (42). Additionally, the response of DHEA to exogenous ACTH administration is notably diminished with age (57).

The concentration ratio of glucocorticoids to DHEAS is closely tied to aging, with a gradual increase. Cortisol has neurotoxic effects by stimulating neuronal degeneration through increased susceptibility to metabolic and vascular injuries, reduction of dendritic length, and cell death possibly associated with apoptosis (33). On the other hand, DHEAS enhances long-term potentiation of neurons and protects from structural damage and functional impairment, promoting glial, and neuronal survival (42). Consequently, the observed increase in the cortisol/DHEAS ratio during aging, leads to enhanced neurotoxicity and probably contributes to the occurrence of age-related neurodegenerative illnesses.

Aldosterone secretion and release from the adrenal cortex declines with aging (58). Basal levels of aldosterone decrease (51, 59), with an associated reduction in renin activity. This characteristic age-related decline in plasma aldosterone refers to men and women as well (60). Despite the limited number of studies and small samples in most of them, the common observation of decreased aldosterone secretion and plasma renin activity in elders, may have significant effects on various aspects related to evaluation and treatment of hypertension in old individuals (58, 61).

Regarding adrenal medullary function, basal adrenaline secretion decreases with age (62). Epinephrine and norepinephrine plasma concentrations become lower or don't change significantly with advancing age (63, 64), so lower secretion from the adrenal medulla in older people is not apparent from plasma concentrations, mainly because of the reduced clearance of these hormones from the circulation (62). Additionally, in cases of acute stress, epinephrine release is mainly lessened in older people, and stimulable elevation in serum catecholamines (as percentages of basal values) also decrease (65, 66). In one study, adrenaline production from the medulla was lower by 40% in elderly healthy men, compared to younger healthy men. Furthermore, adrenaline release in response to stress was increased 33–44% in older men of that observed in young controls. The exact mechanisms responsible for the decrease in adrenaline release from the adrenal medulla observed with aging, have not been fully verified. To some extent, they are possibly related to an age-related decrease in pre-ganglionic nerve activity, reduction in response to pre-ganglionic nerve activity in the adrenal medulla, or possibly depletion in adrenaline synthesis, and storage to the adrenal medulla (62). Conclusively, current evidence shows that adrenal medullary secretion and release of epinephrine are lower in older people, both at rest and during stress (67).

The circadian rhythm is regulated by the hormone melatonin which shows a decrease in levels throughout aging (68). The decrease in melatonin concentrations has been associated with increased incidence of disruption of the normal circadian rhythm in older adults (69). Melatonin is also known to have an immunomodulatory role. While a functional restructuring of activity of the immune system is an integral part of aging, it is not clear if this is associated to changing levels of melatonin (70). Additionally, as a potent antioxidant, melatonin was reported augment cardiovascular function, mostly by its hypotensive effects (71). This notion is further supported in a study on individuals with non-insulin dependent diabetes mellitus, where supplementation with melatonin was found to improve antioxidative defense (72). Of note, melatonin administration improved the circadian rhythm, including sleep and activity at night, but produced no notable changes on daytime activity and naps in Alzheimer type of dementia (73). Finally it has been suggested that melatonin may serve to protect elderly from delirium when given at low doses during acute care (74) (Figure 2).

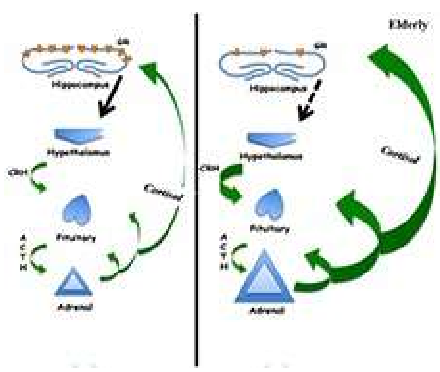

FIGURE 2. Schematic of hypothalamic-pituitary-adrenal axis showing increased cortisol production in the elderly, which may be associated with decreased negative feedback at the hippocampus related to decreased glucocorticoid receptor concentration.

Association of Adrenal Aging on Other Systems

Aging involves a gradual decline in all human functions, including adrenal deterioration. Inevitable clinical sequelae include alterations in body composition, such as loss of density of bone minerals, muscle mass loss, and fat mass increase. These changes may also be related to the endocrine system adjustment to aging (52). Specifically throughout aging, the increase of cortisol levels can cause various effects on multiple systems and adverse changes in older people (Figure 3). As previously stated, elevated cortisol availability has been associated with significant body alterations, leading to the fundamental characteristics of frailty and other functional abnormalities (49). Additionally, while aging, the activity of type 1, 11β-hydroxysteroid dehydrogenase is enhanced in various tissues, such as the central nervous system, skeletal muscles, bones, and skin (46, 75, 76), leading to increased local cortisol formation. This may be clinically correlated with cognitive decline, sarcopenia, osteopenia or osteoporosis, and skin atrophy. Some of the most prominent clinical manifestations of adrenal aging and cortisol increase are briefly discussed below.

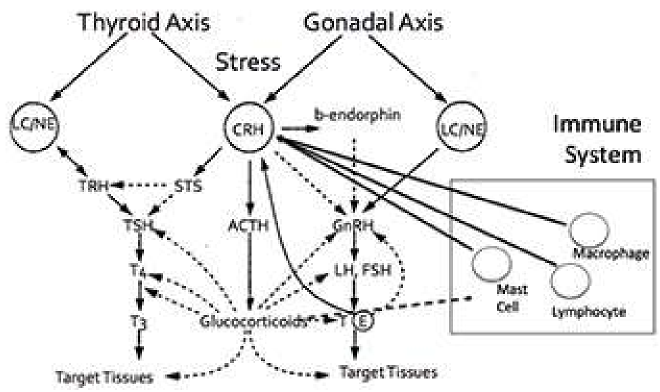

FIGURE 3. The adrenal axis and the end effector, cortisol, demonstrates tight interactions with various other hormonal axes, and systems, including thyroid axes, gonadal axis, and immune system, among others.

Visceral Obesity and Loss of Muscle Mass

Certain characteristic changes in body composition are observed in older persons. These include a decline in total body weight, gradual loss of fat mass (which is normally increasing until the age of about 65), loss of muscle mass, and accumulation of visceral fat (60, 77). Cumulatively, these changes lead to higher total body fat mass and lower total lean mass. Endocrine changes reflected in these alterations include the aforementioned increase in cortisol levels (which is also in part due to the increased production of cortisol by the adipose tissue), insulin resistance, and decline of serum testosterone (32, 78, 79). Total muscle mass reduces by ~30% by the age of 80. This is widely known as sarcopenia, the age-associated loss of skeletal muscle mass and function (79), a phenomenon with important healthcare, and socioeconomic implications. In particular, previous studies have associated muscle loss and fat accumulation with increased urine cortisol secretion (80) and have shown that this decrease of muscle mass and strength is in part due to lipid infiltration of the muscle, resulting in change of muscle quality (81).

Diabetes Mellitus

During the aging process, significant changes of glucose homeostasis include lower levels of insulin and gradually increased resistance to its action (31). Total body composition changes that accompany aging, also promote susceptibility of older people in developing diabetes, by augmenting insulin resistance. As previously mentioned, increase in visceral fat, obesity and alterations in fat to lean muscle mass ratio, affect insulin action, contributing to diabetes pathogenesis in older people (82, 83). In addition, islet β-cells undergo quantitative and qualitative dysfunction, consequently affecting insulin secretion, which is independent of peripheral tissue resistance (84). In fact, in older individuals, β-cell deterioration has a more significant role in the development of diabetes compared to younger adults (32).

Cortisol as a catabolic hormone significantly affects glucose metabolism. Higher cortisol concentrations are associated with insulin resistance and increased fasting glucose (85). It was also demonstrated that the risk of developing diabetes increases with elevated cortisol levels in older people (45). Furthermore, a flatter diurnal slope of cortisol profile (a pattern found in older adults) is related with type 2 diabetes (86).

Osteopenia

One of the most apparent and inescapable effects of aging is a decline in bone mineral density, leading to osteopenia, osteoporosis, and increased risk of fractures. Bone density increases until adulthood, followed by a stable period and thereafter a gradual age-related decline (77). Advancing age impairs bone structure because of an imbalance between bone formation caused by osteoblasts, and bone reabsorption by osteoclasts. Excess of cortisol during aging contributes to the inhibition of bone formation, through stimulation of osteoblast and osteocyte apoptosis (87), extension of osteoclast survival, and suppression of new osteoblast formulation (32). Bone cell glucocorticoid receptors seem to pose an important role to the negative impact of elevated cortisol levels on bone metabolism (88).

Immune Function

Most body systems and organs, including that responsible for immune function, undergo slow, and continuous changes throughout the aging process that ultimately compromises their normal function (89). Among the various factors that change throughout aging and serve critical roles in immunosenescence are a altered capacity for cytotoxicity of natural killer cells, atrophy of the thymus, decreased neutrophil function, reduced number of naive T cells, as well as decreased B cells antibody production (90). It is noteworthy that the HPA axis or stress axis has a critical role in immune system function modulation.

While both adrenal hormones, DHEA and cortisol, modulate immune function, they have opposing effects. Cortisol plays an important role in immunosuppression, while DHEA enhances immune function (89). The immune-enhancement properties of DHEA is associated with changes in its production, which begin decrease after puberty reaching almost 5% circulating concentrations in the elderly compared to pre-puberty (90). On the other hand, cortisol levels remain unaltered, a fact that leads to an imbalance between the two stress hormones (89). The evidence suggests that DHEA increases mitogen-stimulated IL2 release from $CD4^+$ cells and this counters the changes in $CD8^+$ produced by glucocorticoids (91). This suggests that an increase in the ratio cortisol:DHEA may contribute to the decline in immune function observed in the elderly. Thus, DHEA supplementation in the elderly may provide beneficial effects to immune function (92). In addition, stress management as well as acute exercise seem to slow immunosenescence as they improve the cortisol:DHEA ratio (93).

There is ample evidence showing that the effectors of the HPA axis, particularly glucocorticoids, can influence immune function, and immunocompetence via various mechanisms (1). While the data remains conflicting, in general, the elevated levels of circulating cortisol achieved during chronic stress or aging exert immunosuppressive and anti-inflammatory effects. Glucocorticoids do not always suppress immune function, but rather they may act to increase aspects of immune function. None-the-less, hypercortisolemia is associated with augmented function of suppressor T-cells, reduced leukocyte traffic, diminished normal cell-mediated immunity, decreased cytokine production and function, lymphopenia, loss of normal lymph node mass, and thymic involution (94).

Adrenal Aging and Brain Function and Behavior

One of the key questions in neurobiology is how stressful experiences across the lifespan alter the aging process and influences vulnerability to dysregulation of the normal stress response. States of stress induced by psychosocial factors can result in deleterious effects upon the well-being of individuals and predisposing to a variety of disorders. Chronologic age is also a significant predictor of chronic diseases. Psychological stress appears to be a critical aspect in promoting biological aging and earlier onset of age-related disease.

The hippocampus (HC), prefrontal cortex (PFC), and amygdala (AMYG) are highly interconnected key brain regions implicated in stress. Stress induces profound behavioral changes that are paralleled by structural and plastic changes in these areas. HC serves as an important connection between the cortex and hypothalamus, regulating in part, cortisol diurnal rhythm. The HC has an overall inhibitory effect HPA axis activity, serves as a primary central target of stress hormones, and is extraordinarily vulnerable to stress. A key function of the PFC includes the transient storage and manipulation of information to guide subsequent behavior. Dysfunction of the PFC is noteworthy in several psychiatric disorders. The dorsolateral PFC (DLPFC) is important in the conscious regulation of emotion to reduce fear responses and is involved in negative feedback HPA axis regulation. The medial (m) PFC has been implicated in the pathogenesis of MD and SZ and influences HPA axis activity. It has a central role in regulating emotions, reward encoding, and goal directed learning. The mPFC is tightly connected with the DLPFC and limbic areas, particularly the AMYG, which has a central role in the detection of threat and fear. In contrast to the HC and PFC, which decrease in volume after chronic stress, the AMYG increases, which is associated with enhanced anxiety. During emotional challange, the PFC exerts control over the AMYG; successful emotion regulation is associated with increased PFC activity and decreased AMYG activity (28–30).

Stress is a risk factor that affects the physical, mental and social health of individuals through lifespan (95, 96). It is associated with aging-related outcomes at cognitive, emotional, mental, and neurobiological level (97). Over the past decades, there has been an increased research focus on stress and stress mechanisms worldwide due to the aging population and the high morbidity associated with stress-related diseases. Evidence suggests that there is an interplay between chronic stress and the development of depression, anxiety, insulin resistance, dementia as well as cardiovascular diseases (97, 98). Although there is ample evidence about the role of stress in chronic diseases; however the relationship of human biology and environmental factors in terms of causality, remains unclear. It is not feasible to ascertain whether the neurobiological alterations lead to stress-related health outcomes or the environmental stress-related factors result to higher stress levels and neurobiological variations. In other words, cortisol levels are affected by both environmental and endogenous factors.

Aging is accompanied with decrease of and deficiencies in autonomy, health, and social status which entail elevated stress (28). There is a heightened emphasis of the role of the HPA axis in aging and its subsequent effects on the stress-adaptability, stress resistance, and stress-related pathologies (41). The role of HPA axis in stress-related pathologies is well-established mainly due to its sensitivity in both chronic and acute stress, though neurophysiologic variations do exist among individuals and result to differences in aging process, vulnerability, resilience, and stress regulation (41). The variations of the HPA axis by age are in line with the different aging pathways and sub-groups identified in the general population but there is no substantial evidence to determine the consistency of this relationship. Some researchers suggest that older adults experience an anticipated decline in terms of health status which is accompanied by declined cortisol levels (99, 100). On the contrary, according to other studies cortisol levels increased by age (101, 102) while others support that there is no association between cortisol levels and aging (103). There is also the case of elderly that maintain a high level of health status and a normal HPA axis function but they cannot be considered as a representative group of the general population, as well as the elderly chronic patients with poor health status and the most significant deterioration in HPA axis function (41).

Notwithstanding the correlational and not causal relationship between stress, HPA axis and aging, evidence revealed that age-related HPA axis changes affecting the health outcomes of older adults mainly via the diurnal cortisol secretion pathway (78). Elevated adrenal glucocorticoid levels associated with chronic stress have been implicated in alterations in spatial memory, hippocampal function, and cognitive status, in general (104). Negative or traumatic experiences earlier in life, shape the diurinal pattern of cortisol and indicate an individual's level of exposure to chronic stress and subsequently the predisposition for depression, anxiety, and other chronic diseases (41). HPA hyperactivity is linked to higher anxiety levels and increased depressive symptoms. Decreased DHEA and dehydroepiandrosterone sulfate (DHEA-S) release are often found in patients with major depressive disorder (105, 106) while increased DHEA-S is associated with aggressive behavior (13, 107). Furthermore, resilience constitutes a case in point of the interplay between endogenous and environmental stress-related factors and aging and thus it can be used to map the trajectory of HPA axis, stress, and aging (108). Resilience is strongly associated to emotion regulation and social resources (e.g., social support) which in turn affects the HPA axis functioning and vice versa (108, 109). Higher diurinal cortisol levels have been identified in people with low social support and poor resilience which in turn is associated with increased risk for chronic disease and multiple bio-psychosocial implications (41). A healthy aging of brain function is closely related to the quality of health across the life-span and facilitates normal behavior and society integration. The evidence supports that the early prenatal environment has a tremendous impact on later brain aging. Moreover, these early environmental effects in addition to life-style and genetic constructs can have notable effects on age-related brain disorders. Therefore, at health policy context, it is important to develop interventions and programs with the aim to strengthen protective factors such as social support in older adults, so as to increase emotional regulation, reinforce resilience, and decrease the HPA axis dysregulation. Both the aging process, as well as chronic stress have been associated with altered brain function, with consequences in cognitive and emotional processing and an increased vulnerability for brain disorders. Regionally specific changes in brain structure and function associated with chronic stress and aging is associated with increased depression, cognitive changes, anxiety, among others.

Adrenal Aging: Response to Injury and Surgical Stress

Trauma and injury are well-known factors of homeostasis disruption that cause stress to living organisms. Surgical trauma is a controlled and standardized injury in the sterile environment of the operating theater on a patient receiving pharmacologic treatment for pain control with or without anesthesia. Despite this, surgery is a major stressor causing an inflammatory reaction with activation of numerous cytokines, mobilization of cellular response, and a well-defined hormonal response (1). The two most studied systems controlling the injury and stress response are the HPA axis and the sympathetic/parasympathetic autonomic nervous system (2). Mediators, such as pain, anxiety, cholecystokinin, angiotensin II, vasopressin, vasoactive intestinal polypeptide, catecholamines, and proinflammatory cytokines stimulate the secretion of hypothalamic CRH. CRH stimulates the release of ACTH from the anterior pituitary, which in turn stimulates glucocorticoid synthesis and secretion from the zona fasciculata of the adrenal cortex (110). Glucocorticoids are synthetized from a cholesterol moiety and they diffuse readily through the cell membranes to reach the cytosol glucocorticoid receptor of target cells in almost every tissue of the human body. Steroid receptors are inactive by forming a complex with several different molecules of heat shock proteins. Binding of the glucocorticoid molecule to the steroid receptor unbinds the heat shock protein and allows the complex to enter the nucleus where it induces DNA transcription and protein synthesis (111).

Effect of Surgery on the HPA Axis

Researchers have discovered from the early eighties that surgery produces changes in the cortisol circadian rhythm. McIntosh et al demonstrated that in a small group of 10 patients, serum cortisol levels had significantly increased in the second postoperative day after upper abdominal surgery. They also found that this increase was influenced by the type of surgery; high trauma surgery patients had two times greater increase of their serum cortisol levels on postoperative day two in comparison to low trauma patients (112). Ten years later, Naito et al investigated the alterations of the HPA axis in patients undergoing major upper abdominal surgeries such as total gastrectomy, pancreatoduodenectomy, and colectomy. All patients presented a prompt and marked intraoperative elevation of plasma CRH, ACTH, and cortisol levels. Interestingly, both CRH and ACTH had a biphasic change; after this initial peak, they decreased during the first postoperative days to 50% of the preoperative values and returned to normal by postoperative day seven. Intraoperative plasma cortisol levels were more than two times higher than the preoperative levels and progressively dropped down to normal values by postoperative day seven (113). Pooling results from several studies, patients undergoing major surgery present a peak of serum cortisol concentrations from 30 to 45 μg/dL (114). Newer clinical studies compare laparoscopic to open cholecystectomy and laparoscopic to open Niessen fundoplication procedures. Two randomized controlled trials and one prospective study, report that the laparoscopic procedures reduce the acute phase component of surgical injury expressed by serum interleukin 6 (IL-6), C-reactive protein, and prealbumin but do not attenuate the hormonal response expressed by serum cortisol levels (115–117). A study by Siekmann et al. assessing the inflammatory response of patients undergoing colorectal surgery similarly reported that the median serum cytokine concentration of IL-6, IL-8 and IL-10 at one to 6 h after surgery in patients undergoing open surgery was higher when compared to laparoscopic surgery (118). Similarly, a randomized controlled trial by Veenhof et al. found that 2 h after laparoscopic colectomy HLA-DR expression on monocytes was significantly higher and IL-6 level increase was significantly lower compared to open colectomy. However, no difference in serum cortisol levels was evident between the two techniques in both studies (118, 119). Given the diurnal variation of cortisol and the pulsatile secretion of CRH and ACTH it should not be surprising that studies not specifically aiming to investigate the HPA axis may be underpowered to demonstrate differences in postoperative cortisol levels between open to laparoscopic techniques. There is sufficient evidence to support that the extent of surgical trauma influences the secretion of CRH, ACTH, and cortisol during the intraoperative and early postoperative period. Surgery causes, from the moment of the surgical incision, a marked increase in serum CRH, ACTH, and cortisol with all three hormones dropping to normal levels during the early postoperative period.

The HPA Axis of Elderly Patients Undergoing Major Surgical Procedures

In general, basal ACTH secretion, as well as basal and stimulated, cortisol release does not change in the elderly (120). Regardless of age, the cortisol levels are similar in patients with acute myocardial infarction and ACTH stimulation tests showed no difference in cortisol peaks (121). Historic data from autopsy series found that the adenohypophysis is subjected morphological changes in old age undergoing weight reduction and fibrous shrinkage, however, these changes do not correspond to functional degradation (122). Contradictory data come from a Japanese retrospective study of 96 patients with large symptomatic pituitary tumors. Patients over 70 years of age suffered more frequently acute adrenal insufficiency and severe hyponatremia in comparison to younger patients with the authors suggesting that the HPA axis functional reserve is reduced by old age (123).

Old age is not synonymous with incapacity and frailty. Frailty is associated with an increased vulnerability to stressful stimuli, with a decreased ability to maintain a controlled, normal response to intrinsic and environmental stressors, and decreased ability to maintain both physiological, and behavioral homeostasis. Almost 20–30% of the population over 75 years of age is associated with geriatric frailty, which increases notably with advancing age (124). Empirical knowledge dictates that older patients recover slower after major surgical procedures. A large metanalysis of 5,186 patients analyzing surgical outcomes following pancreaticoduodenectomy in elderly patients reported increased post-operative mortality and pneumonia in patients over the age of 75 years, and increased post-operative complications in patients over the age of 80 years (125). Watters et al investigated the recovery of strength in patients older than 70 years of age after major abdominal surgical procedures when compared with patients younger than 50 years of age. Older patients had lower preoperative strength, lower absolute postoperative strength levels, less rapid, and less complete recovery of strength. However, postoperative urine cortisol levels were similar in old and young patients (126). Considering the previous studies older people should not be at an increased risk of adrenal failure. Nevertheless, data from a nationwide study in Taiwan reports that adrenal insufficiency has an incidence in individuals over 60 years old is $92.4/10^5$ of the geriatric population that is six times greater than that observed in the general population. Most of these patients have severe comorbidities, infectious and pulmonary diseases, fluid and electrolyte disorders, and complicated diabetes mellitus (127). Sepsis/SIRS, various drugs, HIV, CMV, and systemic fungal infections are well-known causes of primary adrenal insufficiency (114). A plausible explanation may be that older patients may have a reduced HPA functional capacity due to subclinical secondary adrenal insufficiency from the comorbidities of old age and not the aging process of the adrenal glands.

CONCLUSION

Both normal aging and chronic stress appear to affect the body via shared mechanisms related to glucocorticoid function. The chronicity of both the aging process, particular in relation to alterations in the structure and function of the adrenal gland, and stress can be detrimental to an individual's general well-being. The available evidence supports that the synergy of aging and chronic stress, via their common end-point effector cortisol, can adversely affect the function of numerous vital systems, leading to neural and cognitive changes, osteopenia, diabetes mellitus, visceral obesity, altered immunocompetence, among others.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Impact of Paternal Age on Seminal Parameters and Reproductive Outcome of Intracytoplasmatic Sperm Injection in Infertile Italian Women

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Background: We conducted a retrospective study on a cohort of couples attending the Department of Andrology and Reproductive Physiopathology at Sandro Pertini Hospital in Rome for Intracytoplasmatic Sperm Injection (ICSI)-assisted reproduction programs. Some of the couples included in the study underwent more than one ICSI cycle. Between January 2015 and April 2017.

Objective: To evaluate whether the advancing of the paternal age may have effect on the seminal parameters, thus negatively affecting the embryo formation, development and quality, as well as the pregnancy rate.

Materials and Methods: Five hundred and forty three ICSI cycles were performed on 439 couples undergoing Assisted Reproductive Technologies (ART). Patients were subdivided into three male and three female age groups having similar size:

Men: ≤38 years (M_I), 39–43 years (M_{II}), ≥44 years (M_{III}).

Women: ≤35 years (F_I), 36–40 years (F_{II}),≥41 years (F_{III}).

Discussion and Conclusion: Male age groups did not reveal any statistical significant differences in any age-related semen parameters. We also confirmed a statistical significant increase in the pregnancy rate of couples with older partner age difference and younger female. We found that the advanced male age increases the probability of obtaining one or no type A embryo ($N_A \leq 1$), which was almost doubled in the M_{III} group in comparison with M_I, suggesting a negative effect of male age on the efficacy of the reproductive outcome in terms of a reduced number of type A embryos. Such an effect does not seem related to semen parameters and may deserve further investigations.

Keywords: paternal age, ART, ICSI, infertility, sperm quality parameters

INTRODUCTION

In today's society, economic development and women's growing desire for professional fulfillment has increasingly led to the postponement of parenthood. It is well-known that both the quality and quantity of oocytes is depleted by advancing age. A number of studies have shown that the decline in oocytes is also associated with a reduction in fertility for over 35 years (1, 2). This absolute natural phenomenon accelerates between 36 and 38 years, thereby leading to a rise in the number of infertile women nearing the age of 40 who contacted the assisted reproduction centers with the belief that assisted reproduction technology (ART) is still very effective regardless of its age. While the biological clock determines the end of fertility in women, it does not seem to have a prominent role in men. Male gametogenesis goes on until late in life, according to theory, it enables men to father children even at advanced ages. However, spermatogenesis does undergo both minor and major changes over the years, as reported by the literature. During the 6th decade of life there may be important modifications in hormonal status, sperm characteristics, and histologic and cytologic testicular structure (3–6). At present, there are no legal or biological restrictions on the participation of older men in the assisted reproduction programs. Among the factors affecting the outcome of these techniques, attention is mainly given to female factors and a large body of scientific evidence confirms the importance of them on the reproductive outcome. By contrast, the fewer studies investigating the role played by male partners showed conflicting results (7–11). Given the above, we decided to conduct a retrospective study, from January 2015 to April 2017, on a cohort of couples undergoing assisted reproduction (ICSI). Our objective was to evaluate whether the advancing of the paternal age could have effect on the seminal parameters, thus affecting the embryo formation, development, quality, and the percentage of pregnancy rates.

MATERIALS AND METHODS

Patients

The present study does not require specific approval of the ethics committee as it is a retrospective study requiring a simple "acknowledgment" (protocol 56773/2016) as per the regulation of the Lazio Ethics Committee 2. We carried out a retrospective study on a cohort of couples attending the Department of Andrology and Reproductive Physiopathology at the Sandro Pertini Hospital in Rome for the ICSI-assisted reproduction programs. Some of the couples included in the study underwent more than one ICSI cycle. All couples before, during and after the assisted fertilization path were also supported by a psychologist. From January 2015 to April 2017 we performed 1,181 ICSI cycles on 816 couples (1,026 transfers) undergoing ART. Couples who stopped treatment on their own or due to the risk of Ovarian Hyperstimulation Syndrome (OHSS) (191 cycles, 162 couples), couples whose male partners presented azoospermia or severe oligoasthenoteratozoospermia requiring Fine Needle Aspiration (FNA) (36 cycles, 33 couples) and couples whose female partners are needed, for therapeutic reasons, to cryopreserve all oocytes recovered during pick-up (215 cycles, 161 couples) or embryos achieved (69 cycles, 62 couples) and all female partners with a female disorder (such as endometriosis, reduced ovarian reserve, frequent miscarriages and endocrine ovulatory pathology (127 cycles, 108 couples, 117 transfers) were excluded from this study. The statistical analysis, therefore, includes 543 cycles (439 couples, 523 transfers). In order to assess whether and how male ages affects seminal parameters and reproductive outcome we further subdivided our cases into three male groups and three female age groups having similar size (Table 1).

Table 1. Male and female age groups used in the analysis.

Male age groups			Female age groups		
	Range	*N*		Range	*N*
M_I ≤38	25–38	174	F_I ≤35	24–35	141
M_{II} 39–43	39–43	186	F_{II} 36–40	36–40	210
M_{III} ≥44	44–64	183	F_{III} ≥41	41–47	192

Examination of Seminal Fluid

All patients underwent seminal fluid examination as described by Zerbinati et al. (12) in accordance with the World Health Organization (WHO) (13) standard protocols.

Ovarian Stimulation Protocol

All the female partners completed an ovarian folliculogenesis stimulation protocol with menopausal human gonadotropins, ultrapurified urinary Follicle-Stimulating Hormone (FSH), recombinant FSH and Corifollitropin alfa from day 2 of their menstrual cycle combined with a Gonadotropin Releasing Hormone (GnRh) antagonist from day 6. The initial dosage of gonadotropins was customized for each patient and then varied during stimulation depending on the ovarian response. When the follicular diameter reached 18–20 mm, human Chorionic Gonadotropin (hCG) 10,000 IU was administered subcutaneously. Transvaginal Oocyte Retrieval (TVOR) was performed 36 h after hCG administration. Luteal phase support was performed with progesterone by subcutaneous administration at 50 mg/day (Pleyris) or vaginal delivery at 600 mg/day (Prometrium, Progeffik), from pickup day to at least the pregnancy test, which was usually scheduled 12 days after the transfer. MII oocytes were used for ICSI. Embryo transfer was performed 3 days after oocyte retrieval. The blood sample for the pregnancy test [β-subunit of human Chorionic Gonadotropin (βhCG) assay] was scheduled 12 days after the embryo transfer. βhCG was monitored until the gestational chamber was visible on ultrasound.

Evaluation of Fertilization, Embryo Quality, and Embryo Transfer

Fertilization was evaluated 18 h after ICSI and was considered normal when two distinct pronuclei were evident (14). Embryos were evaluated by invertoscope (Nikon Eclipse TE-2000-U) and the following parameters for the different cleavage stages were recorded: number of blastomeres, blastomere symmetry, the percentage of fragmentation, the presence of multinucleation (up to 96 h of clotting), inner cell mass, trophectoderm, and blastocele 120 h after cleavage. Embryos were embedded in a single culture medium (Sage 1-Step Medium with Human Albumin Solution, Sage, Denmark) in a trigas incubator at 37°C, 6% CO_2 and 5% oxygen (G-185 Trigas, K-System). One to three embryos were transferred for each couple, depending on the patient's clinical history and the degree of embryo development on the second, third and/or fifth day.

Grade A 48 h embryos: 2–4 symmetric blastomeres, ≤10% fragmentation
Grade A 72 h embryos: 6–8 symmetric blastomeres, ≤10% fragmentation
Grade A 120 h embryos: expanded blastocysts (15).

All stages of gamete preparation and handling for both seminology and embryology were performed by a single biologist.

Statistical Analysis

The statistical analysis was performed using GNU-PSPP 0.10.2 (www.gnu.org/software/pspp/). The relationship between couple of parameters in the whole cohort has been evaluated via the Pearson correlation coefficient r. ANOVA test was used to evaluate the statistical significance of differences among age groups. In the case of dichotomous variables such as β^+ test and gestational pregnancies, logistic regression method was adopted to calculate the Odds Ratio (OR) and to evaluate their statistical significance (OR test).

RESULTS

The statistical analysis was conducted on a total of 543 ICSI cycles in 439 couples. Table 2 comprises of the sampled characteristics: the mean age of the female partners was 38 years (range 24–47); 50% (interquartile region Q1–Q3) were between 35 and 42 years old. The mean age of the male partners was 41 years (range 25–64); 50% (interquartile region Q1–Q3) were between 37 and 45 years old. In this cohort 67% of couples had primary infertility and 33% secondary infertility, roughly unchanged as a function of age classes. The prevalence of female, male, couple, or idiopathic diagnoses as a function of the type of infertility on the entire cohort have no statistical significant differences (Table 2). Patients were then divided into three age groups based on male or female (Table 1). Concerning the causes of infertility, for male age, we found for M_I and M_{II} groups about 30% due to male diagnosis, 35% to female, 15% to couples and 20% to idiopathic causes. The M_{III} group depicted roughly the same prevalence for female (35%) and idiopathic (20%) while the male diagnosis dropped below 20% and couple rise up to 28% ($p < 0.01$). Concerning the female age stratifications we found a significant ($p < 0.01$) progressive drop, while that of male diagnosis from 40% (F_I) to 29% (F_{II}) and 9% (F_{III}). Couple (11%, 17%, 32%) and female (31%, 34%, 41%) diagnosis progressively grew with female age ($p < 0.01$) and the fraction of idiopathic causes remained around 17%. The age difference between men and women within this cohort study significantly increased with the rising of male age ($r = 73\%$, $p < 0.01$). An inverse albeit weaker effect was observed for the age difference in relation to the female age ($r = -29\%$, $p < 0.01$). In the male age group (M_I), women were on the average ~1 year older ($p = 0.04$), while in male age groups (M_{II}) and (M_{III}) women were 2 years (M_{II}) and 7.5 years (M_{III}) younger ($p < 0.001$) than the men. When stratifying by female age, men were on average and always older, F_I: 5 years ($p < 0.001$), F_{II}: 3 years ($p < 0.001$), F_{III}: 1.5 years ($p < 0.03$).

Table 2. Statistical description of the sample (543 ICSI cycles–439 couples).

Female age	Years	
ICSI CYCLES: 543		
Mean (Median)	38 (39)	
Range (Q_1-Q_3)	24–47 (35-42)	
σ (ΔQ)	4.2 (7)	
Male age		
Mean (Median)	41 (41)	
Range (Q_1-Q_3)	25–64 (37-45)	
σ (ΔQ)	5.9 (8)	
Type of Infertility	**Type I %**	**Type II %**
	67	33
Diagnosis per infertility type		
Female infertility	33	42
Male infertility	24	23
Couple infertility	23	18
Idiopathic	20	17

Q1–Q3: first and third quartile; σ standard sample deviation; ΔQ interquartile distance. The diagnosis as a function of the type of infertility is reported in the bottom rows.

The seminal parameters of all the 543 cycles are shown in (Table 3).

Table 3. Semen parameters for the male cohort.

	Volume (ml)	Conc. (N/ml $\times 10^6$)	N/Ejac. (N $\times 10^6$)	Progressive motility (%)	Non-progressive motility (%)	Total motility (%)	Abnormal forms (%)
SEMEN PARAMETERS (543 CYCLES)							
Mean	2.7	37	95.6	30	2.2	31.9	87
σ_m	0.1	1.7	4.7	0.7	0.2	0.7	6
Range	0.1–9	0.1–250	0.2–607.5	0–60	0–15	0–60	70–100

The standard uncertainty on the means is shown in parentheses, $\sigma_m = \frac{\sigma}{\sqrt{N}}$ (N indicates the sample number: 543).

The comparison of semen parameters among the three male age groups did not reveal statistical significant difference in any of the age-related semen parameters or in the total number of cycles carried out (Table 4). The multiple regression analysis of the whole cohort shows a weak but statistically significant negative correlation (Pearson coefficient r) between ejaculation volume (V) and male age ($r_{V,\ Age} = -0.12$, $p < 0.05$) (Figure 1) or male Body Mass Index (BMI) ($r_{V,\ BMI} = -0.15$ ($p < 0.01$). Looking at the total sperm count (N) it was negatively correlated with BMI ($r_{V,\ Age} = -0.12$, $p < 0.05$) while its correlation with male age is negligibly weak and not statistically significant.

Table 4. Mean values, standard uncertainty of the mean values, and range for semen parameters in the 3 male age groups.

Male Age (N.)	Volume (ml)	Concentration (N/ml $\times 10^6$)	N/Ejaculate (N $\times 10^6$)	Progressive motility (%)	Non- progressive motility (%)	Total motility (%)	Abnormal forms (%)
	Mean (σ_m) Range	Mean (σ_m) Range	Mean(σ_m) Range	Mean(σ_m) Range	Mean(σ_m) Range	Mean(σ_m) Range	Mean(σ_m) Range
SEMEN PARAMETERS BY AGE GROUP							
M_I 25–38 (174 cycles)	2.8 (0.11) 0.2–8	35.0 (3.1) 0.1–250	92.7 (6.4) 0.2–607.5	30.0 (1.3) 0–60	2.5 (0.3) 0–15	32.2 (1.3) 0–60	86.4(0.5) 65–100
M_{II} 39–43 (186 cycles)	2.7 (0.09 0.1–6	36.7 (2.7) 0.1–230	94.7 (7.6) 0.2–575	29.0(1.3) 0–60	2.1 (0.3) 0–15	31.2 (1.2) 0–60	87.4(0.4) 70–100
M_{III} 44–64 (183 cycles)	2.7 (0.11) 0.2–9	39.4 (3.2) 0.1–210	99.1 (8.7) 0.35–574	30.0 (1.3) 0–60	2.2 (0.3) 0–15	32.4 (1.3) 0–60	86.7(0.4) 70–100

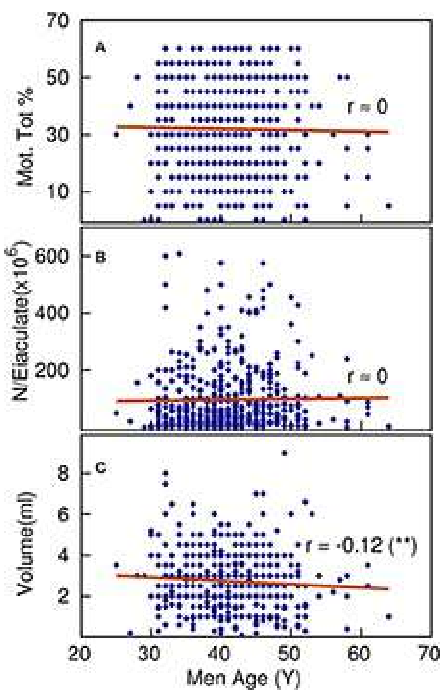

FIGURE 1. The multiple regression analysis of the whole cohort shows negligible, not statistically significant correlation between total motility **(A)** or total sperm count per ejaculated **(B)** and male age, on the contrary a weak but statistically significant negative correlation is found between ejaculated volume **(C)** and male age (p<0.01).

The hormonal profile of male partners is shown in Table 5.

Table 5. Mean values of the hormonal profile.

	FSH (UI/L)	Te (ng/ml)	LH (UI/L)	Inibina B (pg/ml)
$M_I \leq 38$ aa	4.8 ± 1.3	7.4 ± 2.3	4.2 ± 2.3	125 ± 55
M_{II} 39–43 aa	4.7 ± 2.9	6.98 ± 2.08	4.6 ± 2.7	123.5 ± 48
$M_{III} \geq 43$ aa	5.1 ± 1.8	7.01 ± 3.5	4.5 ± 1.09	118 ± 60

We reported data on the semen phenotype for the total male population and the population divided by age group, there was no statistical significant correlation with male age or differences between the age groups (Table 6).

Table 6. Semen phenotypes stratified by male age group in relation to number and percentage of ICSI cycles.

	All %	M_I %	M_{II} %	M_{III} %
SEMEN PHENOTYPE				
Normozoospermia	42	45	37	44
Oligoasthenozoospermia	31	34	29	31
Asthenozoospermia	21	14	28	18
Oligoasthenoteratozoospermia	3.5	5	2	4
Oligozoospermia	1.2	1	2	1
Asthenoteratozoospermia	0.7	1	1	1
Teratozoospermia	0.6	0	1	1

The reproductive outcome of the 543 cycles are shown in (Table 7). The fertilization rate was found to be below 100% in less than the 7% of cases. No statistical significant difference between male age and reproductive outcome parameters was demonstrated. However, the chance of embryo formation was positively correlated with the percentage of progressive sperm motility (r = 0.1,

p = 0.001) and negatively correlated with the percentage of non-progressive sperm motility (r = −0.19, p < 0.001). The correlation between probability of positive pregnancy test (β^+) and clinical pregnancy (cp) have been carried out on the whole cohort as a function of women's and men's age using the logistic regression methods. We found statistical significant correlation between positive pregnancy test and woman's age, with $OR_{\beta+}$ = 0.92 (the 95% confidence interval being $Cl_{95\%}$: 0.88–0.97) and OR_{cp} = 0.92 ($Cl_{95\%}$: 0.87–0.97) implying 8% year-on-year (female age) reduction of the ODDs for positive pregnancy test and clinical pregnancy. By contrast, we did not find any statistical significant correlation between male age and $OR_{\beta+}$ ($OR_{\beta+}$ = 0.98, $Cl_{95\%}$: 0.94–1.01) and OR_{cp} (OR_{cp} = 0.99, $Cl_{95\%}$: 0.95–1.04). The $OR_{\beta+}$ has also been analyzed taking into consideration the age stratified data in female and male age groups. Taking the ODD of F_I and M_I groups as references, we found the F_{II} $OR_{\beta+}$ = 0.79 ($CI_{95\%}$: 0.49–1.28) which is less than 1 but not statistically significant, and F_{III} $OR_{\beta+}$ = 0.44 ($CI_{95\%}$:0.25–76) which is significantly less than 1 (p < 0.05). This implies that, for woman over 41-years, the ODD of a positive pregnancy test is 44% and lesser than the ODD of women younger than 36 years. Looking at the male partners the $OR_{\beta+}$ are less than 1 but not statistically significant (M_{II} $OR_{\beta+}$ = 0.92, $CI_{95\%}$: 0.57–1.50 and M_{III} $OR_{\beta+}$ = 0.70, $CI_{95\%}$: 0.42–1.19). Therefore, the role of male age on the decreasing probability of β^+ cannot be assessed. These findings reflect the mean couple age ($Mean_{age} = 1/2\ M_{age} + 1/2\ F_{age}$): the OR_{cp} analysis as a function of the mean couple age is statistically significant, OR_{tot} = 0.96 ($CI_{95\%}$: 0.93–0.98) pointing out a 4% year-on-year reduction of the ODDs of clinical pregnancy as a function of the couple age. The effect of the age difference between the male and female partner ages ($\Delta = M_{age}$-F_{age}) that gave OR_Δ = 1.04 ($CI_{95\%}$:1.0–1.08) pointing out the rising probability of pregnancy when the female partner is younger is noticeable. In the present study, according to Meijerink et al. (16), we used the probability of obtaining only one or no type A embryo ($N_A \leq 1$) as a negative indicator to evaluate the reduced efficacy of the biological outcome. The probability of $N_A \leq 1$ increases with both male and female age (Figure 2).

Table 7. Mean, standard deviation (σ), median, and range of the reproductive outcome of 543 ICSI cycles.

	Mean	(σ)	Median	Range
Oocytes taken	6.2	(3.5)	6.0	1–25
Oocytes inseminated	3.8	(1.7)	4.0	1–9
Oocytes fertilized	3.7	(1.7)	4.0	1–9
Fertilization rate	99%	(6%)	100%	60–100%
Total embryos obtained	3.2	(1.5)	3.0	1–9
Total embryo rate	88%	(20%)	100%	17–100%
Type A embryos	2.9	(1.5)	3.0	0–9
Type A embryo rate	90%	(23%)	100%	0–100%
Total embryos transferred	2.1	(0.8)	2.0	0–4
Type A embryos transferred	2.0	(0.8)	2.0	0–4
β^+ test	30%			
Clinical pregnancy rate	23%			

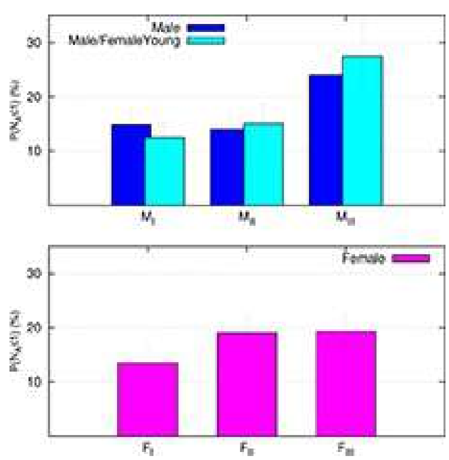

FIGURE 2. (Lower panel) The probability (%) of obtaining $N_A \leq 1$ in the three female age bands (lower panel). The **(Upper panel)** presents the probability (%) of obtaining $N_A \leq 1$ calculated in the three male age groups for the whole cohort (blue) and for a subgroup of couples including only women aged under 41 years (cyan).

This effect was relatively mild and not statistically significant for women but was both evident and statistically significant ($p < 0.01$) for men, ranging from around 15% or less for M_I and M_{II} to about 25% for M_{III}. To quantify the effect of male age on the probability of obtaining $N_A \leq 1$, an OR analysis was conducted. The logistic regression was carried out keeping the M_I ODD as a reference, and it was statistically significant $OR_{NA} = 1.9$ ($CI_{95\%}$:1.06–3.64) between M_{III} and M_I but not between M_{II} and M_I (M_{II} $OR_{NA} = 1.06$, $CI_{95\%}$:0.55–2.03). The $OR_{NA} = 1.9$ means that the ODD for the probability of having $N_A \leq 1$ for the M_{III} males, is almost double respect to that of M_I males ($p < 0.05$). On the contrary for female age stratifications we found the OR_{NA} only slightly larger than 1 but non-statistically significant for F_{II} ($OR_{NA} = 1.51$, $CI_{95\%}$: 0.83–2.74) and F_{III} ($OR_{NA} = 1.53$, $CI_{95\%}$: 0.84–2.80). This finding suggests a major role of male age in increasing the probability of $N_A \leq 1$. In order to further reduce the effect of correlation of male and female ages in the couple we selected a subgroup of couples including only women aged under 41 years (female age groups F_I and F_{II}, 402 transfers) and we performed the same OR_{NA} analysis while keeping the same male age groups as before (Figure 2). We obtained M_{III} $OR_{NA} = 2.66$ ($CI_{95\%}$: 1.34–5.28), which is even larger than the OR_{NA} obtained considering the entire female population. This finding confirms and strengthens the negative effect of male age on the efficacy of the reproductive outcome in terms of a reduced number of A-type embryos that appears not correlated to the female age.

DISCUSSION

The last 40 years have witnessed a profound change in female identity, mainly due to the new role of women in the society. It is now well-known that female reproductive function after the age of 35 years undergoes a physiological aging process that far exceeds that of other organs and tissues. While men experience a gradual decline in fertility from the age of 55–65, this is not comparable with the female menopause, which marks the line between fertility and infertility and has no reproductive purpose (17). Spermatogenesis, in fact, continues until late in life and, according to theory, it enables men to father a child even at a very advanced age. However, it does undergo both minor and major changes as time passes, thereby leading to deterioration in semen parameters, hormone profile and testicular cytological structure (18). The factors affecting ART outcome is mainly related to the influence of female factors, but the few studies investigating the role of male partners offer conflicting results. Most have linked that of the male partner's to exposure to toxic substances (such as ethylene oxide, chemicals in general, solvents, and dithiocarbamates) with the risk of miscarriage (19, 20). In a study of 3,174 women de La Rochebrochard and Thonneau (7) demonstrated a clear negative effect of maternal and paternal age on the risk of miscarriage, by establishing three trends. For women aged 20–29 years, the risk of abortion is relatively low regardless of their partner's age; for women aged 30–34 years, the risk of abortion is higher if their partner is ≥40 years, and for women aged ≥35 years, the risk of abortion increases regardless of their partner's age. The authors concluded that the risk of abortion rises with the increasing age of both partners (7). Further studies have considered the effects of paternal age on the induction of premature births, although the results are inconclusive (8–11). However, the association between advanced paternal age and autosomal dominant disorders and genetic mutations has been extensively investigated (21). There is a body of scientific evidence indicating that genetic factors play an important role in reproductive timing (22). As it is well-known, the placenta is mainly of paternal origin, so if reproductive timing is guided by placental or fetal genes and if mutations in these genes occur most commonly in the gametes of older men, then advanced paternal age could play a decisive role. Zhu et al. (23) conducted a cohort study on the Danish population to investigate any association between paternal age and congenital malformations in the offspring, analyzing data from 71,937 couples between 1980 and 1996 and obtaining diagnoses of possible malformations in the firstborn of these couples from the national register. The authors concluded that men over 45 years have a 4.5-fold greater risk of having a child with trisomy 21 than men under 30 years. In the literature the association between advanced paternal age and the reproductive outcome is still under debate. As the fertilization process involves both partners, it is difficult to eliminate or control the influence of women's age on reproductive potential. To reduce the impact of female factors on reproductive potential, Frattarelli et al. (24) and Luna et al. (25) conducted studies to assess the effects of paternal age on embryonic development and reproductive outcome using donor oocytes. Both groups concluded that advanced paternal age influences the outcome of pregnancy and the percentage of blastocyst formation for men aged >50 years. Conversely, they found no statistical significant correlation between paternal age and the ability of the spermatozoa to penetrate oocyte or the formation of embryos. Luna et al. (25) also reported a statistically significant decrease in the implantation rate, but only in couples in which the male partner was more than 60 years old. Another study by Ferreira et al. (26) evaluated the effects of paternal age on reproductive outcome in 1,024 couples undergoing assisted reproduction cycles (ICSI) by investigating both normozoospermic and oligozoospermic patients. They found that paternal age negatively affects the embryo implantation and pregnancy rate in couples with a sperm concentration of <20 × 10^6/mL. In oligozoospermic patients, the chance of achieving pregnancy dropped by 5% for each 1-year increase in age. In a review of 10 studies, Dain et al. (27) found no correlation between advanced paternal age and fertilization, implantation, pregnancy, miscarriage, and birth rates. Furthermore, no negative effect of paternal age was found on embryonic quality and stage of cleavage (days 2–3). However, there was a statistically significant decrease in the formation of blastocysts with increasing paternal age. In a review, Sharma et al. (28) found that paternal age does not significantly affect miscarriage rate or embryo quality. However, in women aged 30–34 years old, the implantation rate dropped with increasing paternal age and the pregnancy rate was significantly higher with male partners aged <30 years or 30–32 years compared to men aged 36–38 or 39–41 years. Meijerink et al. (16) conducted a retrospective study on 7,051 IVF/ICSI cycles. They did not found any statistically significant difference in pregnancy rate for men aged 35–44 years or for men ≥45 years compared to the control group of men <35 years. They also found no statistically significant effects of paternal age on embryo quality, biochemical pregnancy and spontaneous abortion, and they concluded that paternal age does not influence pregnancy rate in early IVF/ICSI cycles. In the light of literature findings, it seems evident that the influence of paternal age on the reproductive outcome is not unequivocal. The purpose of this study was therefore to evaluate whether increasing age affects sperm quality and hence the reproductive outcome. To this end, we excluded most of the possible female factors known to affect the timing and reproductive outcome (reduced ovarian reserve, endometriosis, recurrent pregnancy loss, etc.). Analysis of the couples' ages and age difference between the male and female partners revealed a positive correlation between age difference and age of the male partner, but a negative correlation between age difference and age of the female partner: the female partner was on average 1 year older than the male partner in the M_I age group, but younger than the male partner in the M_{II} and M_{III} classes. No statistically significant differences were found when analyzing semen parameters in the 543 cycles including after stratification by age of the male partner. From our data, we did not assess any evidence that the increasing male age may affect sperm to such an extent as to compromise semen quality, as also found by Spandorfer et al. (29). This result contrasts with some data reported in other literatures in which the authors found that semen volume, progressive sperm motility and percentage of abnormal forms were significantly lower in older men than in younger subjects (5, 30). These discrepancies demonstrate the complexity of carrying out a study that takes into consideration a significant number of subjects aged over 60 years. A further confounding factor could make the little information available on possible internal and androgenic disorders potentially affecting semen parameters in addition to physiological tissue aging. We found a statistically significant negative correlation between BMI and ejaculation volume but not on semen parameters, confirming the results of Duits et al. (31) and Shayeb et al. (32). In fact, the increase in aromatization activity caused by high concentrations of adipose tissue results in the conversion of testosterone to estrogen; as a consequence, excess leptin causes a drop in testosterone production by Leydig cells, thus altering the functionality of seminal vesicles (33). Furthermore, when stratified by male age, there was a weak but statistically significant positive correlation between the percentage of embryos formed and progressive sperm motility and a weak but statistically significant negative correlation with non-progressive sperm motility. Motility is a fundamental sperm property; its fertilizing capacity depends on chromatinic and mitochondrial integrity (34), both necessary to enable the sperm cell to swim up the female genital tract, penetrate the oocyte and form the male pronucleus. Sperm motility appears to be very important not only for natural fertility but also in assisted reproduction, especially in the most advanced technique, ICSI, which allows fertilization with very few spermatozoa. In this case it is of critical importance to have motile sperm cells, an unmistakable sign of their viability. Kasai et al. (35) demonstrated a higher fertilization and pregnancy rate in patients with higher sperm motility and mitochondrial membrane potential. It is therefore very important to understand the molecular processes underlying sperm motility, as less mobile semen samples can be treated with gene or pharmacological therapies before ART. Our results are in accordance with those of Wu et al. (36) and Begueria et al. (30) who found that paternal age did not significantly affect embryo quality, embryo cleavage stage, or miscarriage rate. However, these authors demonstrated that in women aged 30–34 years, the implantation rate dropped with advancing paternal age and the pregnancy rate was significantly higher for couples with male partners aged <30 or 30–32 years than for male partners in the 36–38 and 39–41 age groups. Concerning these parameters, we did not see a statistically significant effect of age in our data. There was a statistically significant negative correlation between positive pregnancy test and clinical pregnancy rate and age of the female partner, with an 8% year-on-year (female age) drop in the ODD ratio for the chance of getting pregnant. When stratified by female age group, our data showed that the ODD ratio for the probability of positive pregnancy test for women aged ≥41 (F_{III}) is less than half of women ≤35 years (F_I). Our results did not reveal any effect of paternal age on the probability of a positive pregnancy test and clinical pregnancy test in the total cohort sample or when stratified by male age. These results are in agreement with those found in the literature (16, 18, 24, 37, 38). When analyzing the effect on the reproductive outcome of the mean age of the couple and the age difference between the male and female partner we observed that increasing the couple age is significantly related to a reduction in the clinical pregnancy rate. We also confirmed a statistically significant increase in the pregnancy rate in couples with higher partner age difference and younger females. An important issue concerning the efficacy of the biological outcome that emerges from this study is that the probability of achieving none or only one type A embryo increases with both male and female age. This is very evident and statistically significant for the male partner indeed ODD is almost doubled in the M_{III} class in comparison with M_I. More interestingly the negative effect of male age on raising the probability of $N_A \leq 1$, is even more evident when the sample is restricted to the young women couples (female age <41 years): reducing the sample to the couples with the female partner in age groups F_I and F_{II}, the ODD for $N_A \leq 1$ probability for men in age group M_{III} is almost three time larger than M_I. Noticeably a reduction of the quality embryo probability ODD for older men couples is also reported in Meijerink et al. (16) but without statistical significance. It is noteworthy that our finding is a relatively new result strongly supporting some negative effect of male age on the efficacy of the biological outcome, but it does not seem related to any changes in seminal parameters.

AUTHOR CONTRIBUTIONS

RR took part in the conception and design of the study. MG drafted the article and took part in the analysis of the data. MG and AD approved the final version of the manuscript. CarM acquired and analyzed the data. All the other authors have reviewed the manuscript critically.

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REVIEW
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Diabetes and Aging: From Treatment Goals to Pharmacologic Therapy

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Diabetes is becoming one of the most widespread health burning problems in the elderly. Worldwide prevalence of diabetes among subjects over 65 years was 123 million in 2017, a number that is expected to double in 2045. Old patients with diabetes have a higher risk of common geriatric syndromes, including frailty, cognitive impairment and dementia, urinary incontinence, traumatic falls and fractures, disability, side effects of polypharmacy, which have an important impact on quality of life and may interfere with anti-diabetic treatment. Because of all these factors, clinical management of type 2 diabetes in elderly patients currently represents a real challenge for the physician. Actually, the optimal glycemic target to achieve for elderly diabetic patients is still a matter of debate. The American Diabetes Association suggests a HbA1c goal <7.5% for older adults with intact cognitive and functional status, whereas, the American Association of Clinical Endocrinologists (AACE) recommends HbA1c levels of 6.5% or lower as long as it can be achieved safely, with a less stringent target (>6.5%) for patients with concurrent serious illness and at high risk of hypoglycemia. By contrast, the American College of Physicians (ACP) suggests more conservative goals (HbA1c levels between 7 and 8%) for most older patients, and a less intense pharmacotherapy, when HbA1C levels are ≤6.5%. Management of glycemic goals and antihyperglycemic treatment has to be individualized in accordance to medical history and comorbidities, giving preference to drugs that are associated with low risk of hypoglycemia. Antihyperglycemic agents considered safe and effective for type 2 diabetic older patients include: metformin (the first-line agent), pioglitazone, dipeptidyl peptidase 4 inhibitors, glucagon-like peptide 1 receptor agonists. Insulin secretagogue agents have to be used with caution because of their significant hypoglycemic risk; if used, short-acting sulfonylureas, as gliclazide, or glinides as repaglinide, should be preferred. When using complex insulin regimen in old people with diabetes, attention should be paid for the risk of hypoglycemia. In this paper we aim to review and discuss the best glycemic targets as well as the best treatment choices for older people with type 2 diabetes based on current international guidelines.

Keywords: type 2 diabetes, elderly, diabetes-related comorbidities, glycemic targets, glucose lowering drugs

INTRODUCTION

Life expectancy is defined as the average number of years that a newborn is expected to live assuming that current mortality rates remain the same throughout its life. Global average life expectancy has increased by 5.5 years between 2000 and 2016, with the fastest increase since the 1960s, as a consequence of declining number of deaths from infectious causes (1). Latest estimates of life expectancy at birth were of 80.9 years across the 28 European member states (2) and 78.9 years in United States of America (USA) (3). The progressive decline of age-standardized rates of death from non-communicable chronic diseases (NCDs, cardiovascular and respiratory diseases, cancer, and diabetes) registered globally between 2006 and 2016 (4), together with the rising number of people older than 65 years, especially in westernized countries, has led to an increased prevalence of NCDs among elderly, resulting in more years of life spent with morbidity and disability (5).

Diabetes is recognized as an important cause of premature death and disability. In the past three decades the age-standardized prevalence of diabetes has risen substantially in countries at all income levels; 40% of this increase is estimated to result from population growth and aging (6). Therefore, diabetes is one of the most widespread health burning problems in the elderly, which represent a heterogeneous and complex population as it include both newly diagnosed older diabetic patients and patients with long-standing diabetes with onset in middle or early age (7). Consequently, management of diabetes in elderly subjects is particularly complex and challenging for clinicians, due to difficulty in individualizing glycemic targets, treatment strategies, coexisting comorbidities, polypharmacy, and hypoglycemic risk. The aim of this review is to discuss the best glycemic targets as well as the best treatment choices for old people with type 2 diabetes based on current shared international guidelines.

EPIDEMIOLOGY

Type 2 diabetes represents the most common metabolic disease in older adults. According to the latest estimates of the International Diabetes Federation (IDF), diabetes shows a high prevalence in people older than 65 years (8). In 2017, the number of diabetic people aged 65–99 was estimated to be 122.8 million (around 18% of prevalence rate), of whom 98 million had <80 years (65–79 years); these numbers are expected to easily exceed 200 million in 2045 (8). China, United States of America and India are the countries with highest numbers of people older than 65 with diabetes. Similar prevalence rates of diabetes were found in the European Region, reaching values ranging between 14.9 and 25.0% (8). The main reasons imputable to this spreading may be found in the longer life expectancy, the global diffusion of both unhealthy lifestyle habits and environmental pollution (9).

The number of deaths caused by diabetes in the age range of 60–99 years in 2017 was 3,200,000, which represents ~60% of deaths due to diabetes among the age group between 18 and 99 (8). Moreover, elderly diabetic patients are exposed to a higher risk of cardiovascular complications, including peripheral vascular disease, heart disease, and stroke (10), and many geriatric syndromes (from cognitive impairment to urinary incontinence) (11).

PATHOPHYSIOLOGY OF DIABETES IN ELDERLY

Several factors participate in the pathophysiology of diabetes in older age. Chronological age per sè represents a risk factor for many chronic diseases (12). Advanced age leads to the exacerbation of systemic chronic inflammation, oxidative stress, DNA damage, decline of mitochondrial function, cellular senescence, and tissue dysfunction, all conditions which contribute to generate metabolic disorders (13). Indeed, aging is associated with raised levels of pro-inflammatory molecules, including interleukin (IL) 1, IL-6, IL-8, IL-13, IL-18, C-reactive protein, interferons α and β, transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), and serum amyloid (14). Furthermore, the age-related variation of body composition leads to an increase in fat mass, especially visceral adiposity, and an equal decrease in lean and skeletal mass (15). With aging, there is a decline in preadipocyte replication and an expansion of senescent cells in adipose tissue which enhance lipotoxicity and favor the generation of a pro-inflammatory status (16). Moreover, some studies have showed that aging (1) impairs insulin secretion from β-cells in response to endogenous incretins (GIP), (2) is associated with reduced insulin sensitivity, and (3) promotes β-cell death by inducing mitochondrial dysfunction (14). In older subjects, abnormalities in both insulin sensitivity and insulin secretion lead gradually to impaired glucose tolerance and consequently to clinically manifest diabetes. Postprandial hyperglycemia is a characteristic feature of type 2 diabetes in older patients. Therefore, an oral glucose tolerance test should be performed in older subjects with impaired fasting glucose to early detect diabetes, which otherwise could be undiagnosed using fasting plasma glucose alone (7).

DIABETES AND GERIATRIC SYNDROMES

Diabetes onset in elderly usually manifest with vague and not specific symptoms, such as dehydration, dry mouth, confusion, fatigue, lethargy, weight loss, and an increased tendency toward genitourinary infections (17). It has been estimated that 60% of older patients with type 2 diabetes has at least one other comorbid disease, and 40% of these patients has actually no <4 concurrent illnesses (18). Most common type 2 diabetes comorbidities, including cognitive impairment, disability, depression, apathy, urinary incontinence, polypharmacy, hearing, and visual impairment, falls and fractures, fall under geriatric syndromes (19) (Figure 1). With advanced age, malnutrition, physical inactivity, and unwanted weight loss become more frequent. Moreover, elderly diabetic patients are more likely to experience severe or unaware hyper/hypoglycemic episodes and major adverse cardiovascular events (MACE), due to peripheral and autonomic neuropathy. Therefore, a comprehensive geriatric assessment including screening for microvascular complications, cardiovascular risk factors, and geriatric syndromes should be performed at initial diagnosis of diabetes in elderly patients (20).

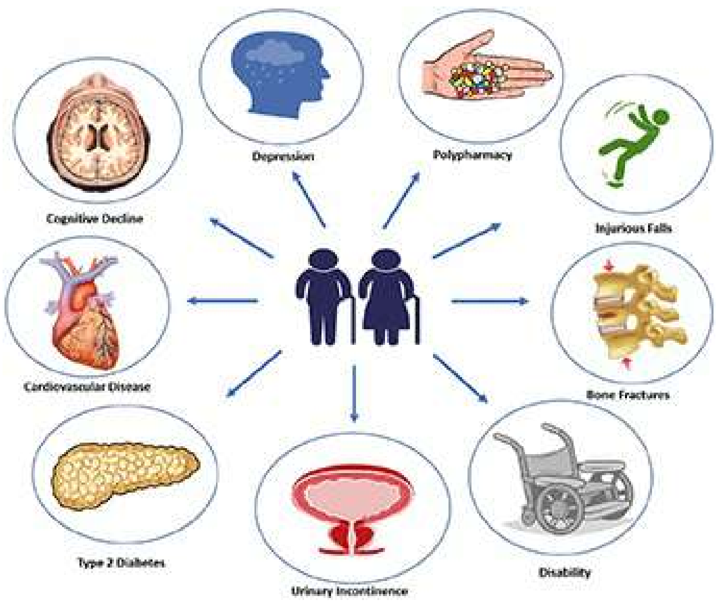

FIGURE 1. Most common clinical conditions associated with aging.

Cognitive Dysfunction and Depression

There is evidence that type 2 diabetes is associated with cognitive dysfunctions. Older diabetic patients have higher risk to develop mild cognitive impairment (MCI), all-cause dementia and Alzheimer's disease (21). Specific mechanisms underlying this association are still unclear; however, main factors involved are vascular dysfunction, high blood pressure, hyperglycemia, hypoglycemic events, insulin resistance, and neuroinflammation (22). Furthermore, depressive and apathic symptoms frequently co-exist with diabetes (23), and some studies have found that combination of diabetes and depression may express a toxic effect on the brain, increasing the risk for dementia (24). In light of this, the American Diabetes Association (ADA) recommends for subjects over 65 years old (with a level of evidence B) a neuro-psychological screening at the initial visit and annually to early detect mild cognitive impairment and depression, by using some specific test (Mini-Mental State Examination, Montreal Cognitive Assessment and Geriatric Depression Scale), and minimizing hypoglycemic events to reduce the risk of MCI (25).

Disability, Fractures and Urinary Incontinence

Type 2 diabetes in elderly is a powerful risk factor for functional limitations, frailty, loss of independence, and disability (26). Moreover, there is evidence that type 2 diabetes increases the risk of fracture risk and secondary hypogonadism, which also contribute to enhance risk of osteoporosis and muscle weakness in men (27, 28). With aging there is a progressive loss of strength and toughness of skeletal and muscle mass which leads to a status of osteo- and sarcopenia. Changes in skeletal muscle protein turnover could accelerate these alterations in type 2 diabetic patients (29), resulting in a greater risk of falling and bone fractures (30). As testosterone decline with advancing age, the assessment of its concentrations may be useful in case of signs and symptoms of overt hypogonadism to better evaluate the risk of fracture in this selected population (31, 32). Indeed, there is evidence that older patients with type 2 diabetes have an increased risk of hip fractures, particularly in insulin-treated patients, and non-skeletal fall injuries (33). A moderate but regular physical activity and a high adherence to Mediterranean dietary pattern showed some benefits in reducing the risk of falls and physical impairments in patients older than 75 years (34, 35). The American Geriatrics Society suggests to interrogate older patients about falls at least every 12 months, examine potentially reversible causes of falls (medications, environmental factors, limiting factors) and perform a complete basic evaluation when an injurious fall occurs (level of evidence III, strength B) (36).

Urinary incontinence is a frequent comorbidity of diabetes, although it is usually not-reported by patients (37). Therefore, according to the American Geriatrics Society, physicians should always perform an annual screening for urinary incontinence which may be an important cause of social isolation, depression, falls, and fractures (level of evidence III, strength A) (36).

Overtreatment and Polypharmacy

Both overtreatment and polypharmacy are very common among frail older diabetic subjects. The prevalence of polypharmacy regimen, defined as the use of more than 5 medications, increases with age. Results from a Dutch study revealed that 64 persons (20%) out of 319 type 2 diabetic patients aged ≥70 years were overtreated and frail (38). Furthermore, one-quarter of US older diabetic adults are on potential overtreatment for tight glycemic control using glucose-lowering medications at high risk of hypoglycemia (39). In a cohort of 8,932 adults with diabetes, 78% of patients had polypharmacy, which was more likely associated with age ≥60 years, female sex, and coexisting chronic diseases (40). Polypharmacy in older diabetic patients may produce detrimental effects mainly due to increased risk of drug-drug interactions and adverse side effects (41). However, a deintensification rather than intensification of pharmacological therapy should be advisable in diabetic patients in older age, in consideration of both benefits and risks associated with complex therapeutic regimens. Moreover, older adults with diabetes should annually update the list of used medications for their own clinicians (level of evidence II, strength A) (36).

GLYCEMIC CONTROL

Older patients represent a very heterogeneous and challenging population concerning diabetes care and treatment. While treating diabetes in elderly, clinicians should be always aware of maintaining a good quality of life. Patient-centered glycemic targets are needed in order to achieve the glycemic control avoiding dangerous or extreme glucose excursions. Elderly patients are highly vulnerable to hypoglycemic events, as a consequence of progressive age-related decrease in β-adrenergic receptor function. Indeed, hypoglycemia in older age has been associated with an increased risk to develop cognitive impairment, dementia, all-cause hospitalization, and all cause mortality (42–44). Use of insulin or insulin secretagogues, polypharmacy, coexisting comorbidities, renal insufficiency, dehydration, impairment of counter-regulatory responses represent the main predisposing risk factors for hypoglycemic episodes (45). Assessment of potential risk factors for hypoglycemia is an important part of the clinical management of older diabetic subjects. Moreover, both patients and caregivers have to be trained and well-educated on the prevention, detection, and treatment of hypoglycemic events (11). On the other hand, both untreated or undertreated hyperglycemic events should be avoided in old people, given the higher risk of dehydration, dizziness, falls, and long-term mortality (46).

The paucity of randomized controlled trials (RCTs) for diabetes treatment in older adults does not allow to clearly establish the most appropriate therapeutic goals in the elderly. Three major high-profile trials (ACCORD, VADT, and ADVANCE trials) (47–49) conducted on type 2 diabetic people aged around 60 years old showed that achieving tight glycemic control (HbA1c < 6% or < 6.5%) was not associated with improvements in cardiovascular outcomes, and one of them (47) has been stopped earlier because of increased mortality in the intensive glucose control arm (number of death in intensive vs. standard therapy, 257 vs. 203, HR 1.22; $P = 0.04$) and increased hypoglycemic events (538 vs. 179, $P < 0.001$). On the other hand, a large observational study reported that an HbA1c level > 8% was associated with increased risk of all-cause, cardiovascular, and cancer mortality in older adults with diabetes (50). Actually, the best glycemic target to achieve for elderly diabetic patients is still a matter of debate (51). However, there is agreement on tailoring glycemic goals in function of patient's life expectancy, diabetes duration, functional status, existing comorbidities, and pursuing moderate (HbA1c between 7 and 8%) rather than tight control (52) in old diabetic patients.

WHAT DO CURRENT INTERNATIONAL GUIDELINES SAY ON GLYCEMIC GOALS?

Table 1 summarizes the glycemic goals for elderly affected by diabetes according different international guidelines. The current Standards of Medical Care in Diabetes 2019 released by American Diabetes Association (ADA) indicate an HbA1c goal < 7.5% for healthy older adults with intact cognitive and functional status and a fasting or pre-prandial glucose between 90 and 130 mg/dL, whereas less stringent targets (HbA1c < 8.0–8.5%) may be advisable for frail older adults with limited life expectancy, with fasting glucose level between 100 and 180 mg/dL (25). These therapeutic objectives are in line with those for adults older than 65 years indicated by American Geriatrics Society (HbA1c ranging between 7.5 and 8%), which suggest to determine HbA1c at least every 6 months, or more frequently if needed (36). Beyond tailored glycemic goals, ADA highlights the importance of controlling any other cardiovascular risk factor with an appropriate lipid-lowering, anti-platelet, and anti-hypertensive therapy.

Table 1. Glycemic targets in elderly patients according to the current international guidelines.

International Guidelines, year	HbA1c goal for most healthy older adults with intact cognitive and functional status	HbA1c goal for most frail older adults, with multiple comorbidities and limited life expectancy
ADA, 2019	<7.5%	<8–8.5%
AGA, 2013	7–7.5%	7.5–9%
AACE, 2018	≤6.5%	>6.5%
ACP, 2018	7–8%	No specific target but minimizing symptoms related to hyperglycemia

ADA, American Diabetes Association; AGA, American Geriatrics Association; AACE, American Association of Clinical Endocrinologists; ACP, American College of Physician.

Differing from ADA, the American Association of Clinical Endocrinologists (AACE) advises an HbA1c goal of 6.5% or lower for most patients without history of cardiovascular diseases (CVD) as it can be safely achieved, whereas, a broader HbA1c target (>6.5%) is suggested for older patients with concurrent serious illness, high risk of hypoglycemia, and limited life expectancy, as the patient does not experience characteristic hyperglycemic symptoms (polydipsia, polyuria, polyphagia) (53).

On the other hand, the American College of Physicians (ACP) suggests more conservative goals (HbA1c levels between 7 and 8%) for most older patients, and a less intense pharmacotherapy when HbA1c ≤ 6.5% (54). Moreover, for patients over 80 years old and with important serious chronic diseases (dementia, cancer, end-stage kidney disease, respiratory, and heart disease) clinicians should focus on minimizing symptoms related to hyperglycemia and avoiding an HbA1c target in patients with a life expectancy <10 years (54). Despite discrepancies in international guidelines (55), the mantra that every physician should follow could be resumed in “treat the patient, not the HbA1c level” (56).

DIABETES TREATMENTS

Studies comparing the effectiveness of anti-diabetes drugs in elderly are lacking, due to the exclusion of older diabetic adults from RCTs, given the high number of comorbidity and their enhanced cardiovascular risk. Every therapeutic strategy should be chosen considering age, health status, self-manageability, cognitive and nutritional status, and comorbidities (Table 2). Generally, in older adults at higher risk to experience hypoglycemic events, medications with low risk of hypoglycemia should be preferred. Furthermore, it is advisable to simplify poly-pharmacological regimens in order to reduce adverse effects and achieve most appropriate glycemic goals. The latest consensus on the management of hyperglycemia in type 2 diabetes of the ADA and the European Association for the Study of Diabetes (EASD) (57) recommends to use drugs with proven cardiovascular benefit in patients with established clinical cardiovascular disease. Anti-hyperglycemic agents considered safe and effective for type 2 diabetic older patients can be divided in oral and injectable drugs (Table 3).

Table 2. Most frequent clinical phenotypes in elderly with suggested HbA1c target and glucose-lowering treatment.

Phenotype	Comorbidities	Diabetic complications	Glycemic target	Glucose-lowering treatment
75-year old men HbA1c 7.2% Treated with metformin 1,500 mg/day	Hypertension	None	HbA1c <7.0%	Consider to titrate metformin or add a DPP-4 inhibitor
78-year old woman HbA1c 7.6% Treated with metformin 2000 mg/day	Heart failure (NYHA class III) Osteoporosis CKD (GFR 48)*	Peripheral neuropathy	HbA1c <7.5%	Suspend metformin Consider to start a SGLT2-inhibitor and in second instance a GLP-1RAs or a DPP-4 inhibitor
81-year old men HbA1c 8.4% Treated with Glargine U/day 26	Cerebrovascular disease MCI CKD (GFR 38)* Prostate adenoma	Diabetic ulcer of the right foot	HbA1c <8.0%	Consider to add a GLP-1 RAs (liraglutide, lixisenatide or dulaglutide) or a DPP-4 inhibitor, or to switch to a fixed ratio combo of basal insulin and GLP-1RA
80-year old woman HbA1c 8.7% Treated with a combo of metformin and sulphonilurea 800 + 5 mg/day	Metastatic breast cancer CKD (GFR 29)* Coronary heart disease Recurrent symptomatic hypoglycemia Wasting syndrome	Autonomic neuropathy	HbA1c <8.5%	Suspend metformin and sulphonilurea. On the basis of SBGM, consider to start pioglitazone or a DPP-4 inhibitor or a basal insulin

**GFR is estimated as mL/min/1.73 m^2 of body surface.*
CKD, chronic kidney disease; DPP-4, dipeptidyl peptidase-4; GFR, glomerular filtration rate; GLP-1 RAs, glucagon-like peptide-1 receptor agonists; MCI, mild cognitive impairment; SBGM, self blood glucose monitoring; SGLT2, sodium-glucose co-transporter 2.

Table 3. Glucose-lowering medications available in Europe with specific characteristics to drive the treatment choice for old people with type 2 diabetes.

Anti-hyperglycemic class	Mechanism of action	General characteristics	Potential side effects	Contraindications
Biguanides *Metformin*	Insulin sensitizer agent, lowering glucose concentration by reducing hepatic gluconeogenesis	First line agent in type 2 diabetes. Good efficacy, low cost, no risk of hypoglycemia	Gastrointestinal symptoms, rare lactic acidosis	GFR* < 30 Dose reduction if GFR 30–45
Thiazolidinediones *Pioglitazone*	Insulin sensitizer agent, influencing transcriptional processes by activation of PPAR-γ	Good efficacy, low cost, no risk of hypoglycemia	Weight gain, fluid retention, increased risk of bone fracture and bladder cancer	CHF (NYHA class III-IV), DKA
Sulfonylureas *Glibenclamide* *Glicazide* *Glimepiride* *Glipizide*	Insulin secretagogue agents, acting on SUR subunit of ATP-sensitive K+ channels in pancreatic beta cells	High efficacy, low cost. Short-acting ones preferred in older patients	Hypoglycemia, weight gain	Severe kidney or liver disease. Long-acting ones should not be used in elderly
Meglitinides: *Netaglinide* *Repaglinide*	Insulin secretagogue agents, enhancing early phase of insulin secretion	High efficacy in lowering postprandial glucose levels, low cost. Safe in advanced renal disease with dose adjustment	Hypoglycemia, weight gain	DKA, adrenal insufficiency, hypopytuitarism
DPP-4 inhibitors *Alogliptin* *Linagliptin* *Sitagliptin* *Saxagliptin* *Vildagliptin*	Incretin enhancer agents, they inhibit the DPP-4 enzyme extending GLP-1 life-time, leading to increased insulin secretion and decreased glucagon secretion in a glucose dependent manner	Intermediate efficacy, neutral effect on weight, well-tolerated, no risk of hypoglycemia in monotherapy, proven cardiovascular safety, intermediate cost	Potential risk of pancreatitis. Saxagliptin is associated with higher risk of heart failure hospitalization	Previous episode or risk of pancreatitis. Dose adjustment in moderate to severe kidney disease except for linagliptin. Saxagliptin is contraindicated if GFR* < 15
SGLT2 inhibitors *Canagliflozin* *Dapagliflozin* *Empagliflozin*	Glycosuric agents, they inhibit the Na/Glucose renal cotransporter on kidney proximal convoluted tubule, increasing urinary glucose concentration, and favoring osmotic diuresis	High efficacy, reduced body weight and blood pressure, no risk of hypoglycemia, benefit on cardiovascular and renal outcomes, high cost	Mycotic genital infections, de-hydration, orthostatic hypotension, increased risk of DKA, lower extremities amputations (canaglifozin), bone fracture	GFR* ≤ 30. If used with diuretics dose adjustment is needed
GLP-1RAs short-acting *Exenatide* *Lixisenatide* GLP-1RAs long-acting *Albiglutide* *Dulaglutide* *Exenatide LAR* *Liraglutide* *Semaglutide*	Incretin analogs, activating GLP-1 receptors, thus promoting insulin secretion and decreasing glucagon secretion in a glucose dependent manner, slowing gastric emptying and favoring sense of satiety	High efficacy, no risk of hypoglycemia, weight loss, once-daily or once weekly injection, benefit on cardiovascular outcomes (liraglutide, semaglutide, and albiglutide), high cost	Nausea, vomiting, diarrhea, modestly increase heart rate, potential risk of pancreatitis and thyroid cancer, gallbladder stones	Previous episode or risk of pancreatitis, thyroid cancer, multiple endocrine neoplasia syndrome type 2 (MEN 2), severe kidney disease or dialysis (liraglutide and dulaglutide can be used until GFR* > 15)
Long acting insulin analog *Degludec* *Detemir* *Glargine*	Basal recombinant insulin analogs activating insulin receptor, lowering glucose levels	Very high efficacy, once-daily injection, frequent dose adjustment for optimal efficacy, high cost	Weight gain, hypoglycemia, lipoatrophy, injection site reaction	Hypersensitivity to insulin or its excipients
Short acting insulin analog *Aspart* *Glulisine* *Lispro*	Pre-meal recombinant insulin analogs activating insulin receptor, lowering glucose levels	Very high efficacy, high risk of hypoglycemia, multiple daily frequent dose adjustment for optimal efficacy, high cost	Weight gain, hypoglycemia, lipoatrophy, injection site reaction	Hypersensitivity to insulin or its excipients
Ultra rapid acting insulin analog *Faster aspart*	Pre-meal recombinant insulin analogs activating insulin receptor, lowering glucose levels	Very high efficacy, high risk of hypoglycemia, multiple daily frequent dose adjustment for optimal efficacy, high cost	Weight gain, hypoglycemia, lipoatrophy, injection site reaction	Hypersensitivity to insulin or its excipients

GFR is estimated as mL/min/1.73 m^2 of body surface.

CHF, chronic heart failure; DKA, diabetic ketoacidosis; DPP-4, dipeptidyl peptidase-4; Exenatide LAR, exenatide long acting release; GFR, glomerular filtration rate; GLP-1 RAs, glucagon-like peptide-1 receptor agonists; PPAR-γ, peroxisome proliferating activated receptor-γ; SGLT2, Sodium-glucose co-transporter 2; SUR, sulfonylurea receptor.

Oral Anti-hyperglycemic Drugs

Metformin is the first-line medication recommended in the management of type 2 diabetes. It reduces both insulin-resistance and hepatic gluconeogenesis, lowering glucose concentrations without increasing hypoglycemic risk. The starting dose is of 500 mg once or twice a day to be assumed with meals up to 2,500 mg/day at the maximum dose. Moreover, a once daily extended-release formulation of metformin is now available, which is associated with a better gastrointestinal tolerability profile and patients' compliance. As it is excreted by the urine, a good glomerular filtration rate is needed (58). Therefore, a dose reduction has to be considered if glomerular filtration rate (GFR) is between 30 and 45 mL/min/1.73 m^2, while discontinuation is recommended if GFR < 30 mL/min/1.73 m^2 (59). The main adverse effects described are commonly gastrointestinal symptoms and very rarely lactic acidosis. It is a safe and effective anti-hyperglycemic drug, with low cost, and minimal risk of hypoglycemia. Nevertheless, it should be carefully used under conditions of congestive heart failure and hepatic dysfunction, which could increase the risk of lactic acidosis (25).

Thiazolidinediones also act as insulin sensing agent influencing transcriptional processes by activation of peroxisome proliferator-activated receptor-γ (PPAR-γ). Pioglitazone is the only one remaining drug of this class, as it has proven to be safe in the presence of cardiovascular disease (60). It is characterized by good efficacy, low cost, and no risk of hypoglycemia when used in monotherapy. It can be used even in case of low GFR value (61) starting from the lowest dose of 15 mg to the maximum dose of 45 mg with meals. Pioglitazone is associated with weight gain and fluid retention, so that it is contraindicated in case of congestive heart failure (NYHA class III, IV). Furthermore, it is not advisable to use the drug in older person at risk for falls because it has proven to increase risk of non-osteoporotic bone fractures (62). Finally, it is contraindicated in patients with or at high risk for bladder cancer (63).

Sulfonylureas are an insulin secretagogue class, which act by favoring β-cells membrane depolarization and consequently insulin secretion. They are characterized by high glucose lowering efficacy and low cost, but they should be used with extreme caution because of the high risk of hypoglycemia and weigh gain. Short acting ones with lowest hypoglycemic risk, such as gliclazide, should be preferred in older diabetic patients, when initial therapy with metformin is contraindicated or not tolerated (64). By contrast, long acting sulfonylureas, as glibenclamide, are considered inappropriate in elderly diabetes management.

Metiglinides are short-acting insulin secretagogue agents, that enhance early phase of insulin secretion at meals, lowering postprandial glucose levels. They present lower risk of hypoglycemia than sulfonylureas, since their activity is dependent on the presence of glucose (20). Repaglinide is the most effective agent of this class, with a moderate effect on weight gain. Use of repaglinide may be indicated for elderly patients with type 2 diabetes because of the low risk of hypoglycemia, high efficacy on postprandial hyperglycemia, and safe use in renal impairment (65).

Dipeptidyl peptidase 4 (DPP-4) inhibitors belong to the class of incretin enhancer agents. They inhibit the DPP-4 enzyme, thereby extending the life-time of GLP-1 and increasing insulin secretion in a glucose dependent manner. Drugs in this class are generally well-tolerated in older people, with neutral effect on body weight and very low risk of hypoglycemia (66, 67). DPP-4 inhibitors have proven to be effective in reducing baseline HbA1c levels and fasting plasma glucose (68). Moreover, a study of 80 elderly diabetic patients treated with oral glucose-lowering drug (DPP4-inhibitors or sulfonylureas) for at least 24 months showed that patients using DPP-4 inhibitors had better sarcopenic parameters (fat-free mass, skeletal muscle mass, and related indices, muscle strength, and gait speed) as compared with those receiving sulfonylureas (69). The cardiovascular safety of this class of agents has been confirmed by several randomized controlled trials (70–74). Alogliptin, saxagliptin, sitagliptin, and linagliptin (70–74) have proven to neither increase nor decrease risk of the combined major adverse cardiovascular events (MACE) in type 2 diabetic patients with established cardiovascular disease. However, in the SAVOR-TIMI 53 study (72), saxagliptin, showed a 27% increased risk of hospitalization for heart failure (HF) among patients with elevated levels of natriuretic peptides, previous heart failure, or chronic kidney disease, as compared with placebo (75). In the EXAMINE trial, patients with type 2 diabetes and recent acute coronary syndromes assigned to alogliptin had an increased, although non-statistically significant, rate of HF hospitalization when compared to the placebo group (76). Recently, in the TECOS trial, sitagliptin showed neutral effects on cardiovascular risk without any significant risk of HF hospitalization when compared with placebo in patients aged ≥75 years with well-controlled type 2 diabetes and cardiovascular disease (77). Moreover, data from the TECOS trial report that sitagliptin is not associated with a higher fracture risk, major osteoporotic fractures, or hip fractures (78). Therefore, DPP-4 inhibitors may be considered as an effective and safely treatment option for older patients with type 2 diabetes (79).

Sodium-glucose cotransporter 2 (SGLT-2) inhibitors are the latest marketed oral anti-hyperglycemic agents in diabetes management. These molecules act with an innovative and different mechanism of action: they inhibit Na/glucose renal cotransporter on kidney proximal convoluted tubule, increasing urinary glucose concentration, and favoring osmotic diuresis (diuretic effect). Beyond glucose lowering efficacy, SGLT-2 inhibitors have also beneficial effects in reducing body weight and blood pressure. Their use is permitted until GFR ≥ 30 mL/min/1.73 m^2, due to safety concerns and lack of dedicated study in diabetic population with severe chronic renal disease. If SGLT-2 inhibitors are used in combination with diuretics, lowering the dose of diuretics is needed to minimize the risks of hypotension and dehydration (79). SGLT2-inhibitors are generally well-tolerated in older adults, except for increased risk of mycotic genital infections in both sexes. There is evidence from cardiovascular outcome trials (80, 81) that this class has beneficial effects in reducing the composite endpoint of cardiovascular deaths, non-fatal myocardial infarction and non-fatal stroke as compared with placebo in patients with type 2 diabetes and high cardiovascular risk. Similarly, in the multinational, observational CVD-REAL study, new users of empaglifozin, canaglifozin, and dapaglifozin reported lower risk of cardiovascular mortality, MACE and hospitalization for heart failure as compared with new users of other glucose-lowering drugs (82). Moreover, a subgroup analysis of the EMPA-REG OUTCOME study showed a significant reduction in the risk of MACE especially in patients older than 65 years treated with empaglifozin (80). Based on these results, ADA and EASD recommend their use in patients with established or at high risk of cardiovascular disease (57). In the respective RCTs designed to test the efficacy and safety of SGLT-2 inhibitors on renal outcomes (83, 84), both empagliflozin and canagliflozin use was associated with reduced risk of sustained loss of kidney function, attenuated GFR decline, and a reduction in albuminuria, which supports a possible renoprotective effect of this drugs in people with type 2 diabetes. More recently, treatment with dapagliflozin, compared with placebo, produced a significant 24% risk reduction in renal composite events, namely ≥40% decrease in eGFR below 60 ml/min/1.73 m^2 of body-surface area, new end-stage renal disease, or death from renal or cardiovascular causes (85). Conversely, on May 2015 the Food and Drug Administration released a warning relative to an increased risk of diabetic ketoacidosis (DKA) associated with use of SGLT-2 inhibitors (86), on the basis of a comparative evaluation with DPP-4 inhibitors on a cohort of more than 140,000 type 2 diabetic patients (87). The increased incidence of DKA related to SGLT2-inhibitors may be probably related to the non-insulin-dependent glucose clearance, hyperglucagonemia, and volume depletion (88). Therefore, although this class has many beneficial effects on cardiovascular and renal outcomes, caution is needed using SGLT2 inhibitors in elderly because of increased risk of genital infections, dehydration, orthostatic hypotension, lower extremities amputations, and bone fracture (89, 90).

Injectable Anti-hyperglycemic Drugs

Glucagon-like peptide 1 receptor agonists (GLP-1RAs) are innovative and pleiotropic drugs that act by promoting insulin secretion and reducing glucagon secretion in a glucose dependent manner and favoring weight loss. As they use the injectable way of administration, they require neuro-psychological and physical integrity. GLP-1RAs are highly effective in lowering glucose levels, with minimal risk of hypoglycemia (91, 92). Recently, a phase III RCT showed the superiority of lixisenatide as compared with placebo in reducing HbA1c levels and postprandial hyperglycemia in patients ≥70 years uncontrolled on their current antidiabetic treatment (93). The main adverse effects associated with GLP-1RAs use consist of nausea, vomiting, diarrhea, and an increase in heart rate (94). Furthermore, there is strong evidence from RCTs (95–97) that these drugs can reduce the risk of MACE in type 2 diabetic patients with high cardiovascular risk. Results from preclinical studies showed also favorable effects of GLP-1RAs on neuronal protection and cognitive performances (98, 99). Randomized controlled trials assessing effects of incretin therapy on cognitive function and Alzheimer's disease in humans are currently ongoing. If these benefits will be confirmed, use of GLP-1RA may be a helpful option even in patients with mild cognitive impairment.

Free and fixed-ratio combinations of GLP-1RAs and basal insulin formulations have been approved by regulatory agencies to potentiate antihyperglycemic effects and glycemic control in type 2 diabetic patients (57, 100). At the moment, two fixed-ratio combinations, insulin glargine plus lixisenatide (IGlarLixi) and insulin degludec plus liraglutide (IDegLira), have been approved for treatment of type 2 diabetes (101). A recent analysis compared effectiveness of fixed-ratio combination iGlarlixi vs. sequential administration of iGlar + Lixi in glucose control in type 2 diabetic patients (102). IGlarLixi was associated with significantly higher HbA1c reductions, weight loss and number of patients reaching HbA1c target despite lower insulin doses, with similar rates oh hypoglycemic events and lower rates of gastrointestinal adverse events. A meta-analysis of 26 RCTs have shown a mean reduction of 0.47% in HbA1c level associated with a mean weight loss of 2.5 Kg favoring the insulin/GLP-1RA combination as compared with other injectable anti-diabetes treatments, with no increased risk of hypoglycaemia (103). Moreover, when compared with intensive insulin therapy, either free or fixed combination of GLP-1RA and basal insulin led to a greater mean decrease of 0.53% in HbA1c level, a higher proportion of patients at HbA1c target of < 7% and reduction in body weight (104). Based on this evidence, combination strategies, either free or fixed, represent a good option for intensifying basal insulin therapy in patients with type 2 diabetes who need amelioration of glycemic control, without increasing the risk of hypoglycemia and weight gain (104).

Insulin remains the most effective drug for type 2 diabetes (105). The main limitations of insulin therapy are the risk of hypoglycemia and weight gain, although it can be administered at any GFR value. Insulin therapy requires patients' autonomy, intact visual, motor, and cognitive ability in diabetes management (25). Since its discovery in 1921, several and innovative insulin formulations have been developed. Insulin glargine (U100 or U300), degludec (U100 or U200), and detemir represent long acting insulin analogs which provide daily basal insulin profiles (106). A recent meta-analysis reported that insulin glargine U300 was as effective as glargine U100 in type 2 diabetic patients aged >65 years, with a reduced risk of nocturnal hypoglycemia (107). Compared with human insulin neutral protamine Hagedorn (NPH), long-acting insulin analogs have a longer duration of action and a fatter pharmacokinetic profile, with a reduced risk of hypoglycemia (106). Therefore, the newer basal insulins should be preferentially used in diabetic elderly, where they may be indicated as starting insulin therapy. Prandial rapid (aspart, lispro, glulisine) and ultra-rapid acting (faster aspart) insulin analogs used at mealtime can be combined with basal insulin to sooner improve and intensify glycemic control (108). However, both basal and prandial insulin require frequent titration to achieve the best anti-hyperglycemic effects. Patients on enteral or parenteral nutrition may require frequent glucose monitoring (intervals of 4–6 h) to better titrate the insulin dose and to avoid hypo- and hyperglycemic events (64). Caution is needed in insulin titration because a simple error can easily precipitate major hypoglycemic episodes, leading to falls, and bone fractures (109). Alternatively, premixed insulin regimen, eliminating the challenge of mixing insulin, may have a role in elderly patients who have regular eating habits, with similar efficacy as compared with basal bolus therapy (110). Therefore, use of insulin therapy in elderly patients often requires the assistance of a caregiver if patients' abilities are limited.

CONCLUSIONS

Older adults with type 2 diabetes represent a complex and heterogenous age group. Managing diabetes in older age remains an important clinical challenge for all physicians, either primary care providers or specialists. As older diabetic patients present frequently frailty and/or multiple comorbidities, an individualized patient-centered glycemic target is needed in order to achieve a glycemic control avoiding dangerous hypo- and hyperglycemic events. A comprehensive geriatric assessment should be performed at diagnosis of diabetes to better understand cognitive, visual and motor abilities, and coexisting comorbidities. In the choice of anti-hyperglycemic strategies, drugs with proven tolerability, safety, and minimal hypoglycemic risk should be preferred. Anti-diabetes treatment regimens in elderly must be simple, sustainable, and safe to best mirror patients' preferences, wishes, and needs.

AUTHOR CONTRIBUTIONS

GB, MIM, KE, and DG conceived the manuscript. ML, GB, and MIM drafted the manuscript. JM, KE, and DG reviewed and edited the manuscript. All authors gave the approval to the final version of the manuscript.

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REVIEW
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Overt and Subclinical Hypothyroidism in the Elderly: When to Treat?

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Hypothyroidism is characterized by increased thyrotropin (TSH) levels and reduced free thyroid hormone fractions while, subclinical hypothyroidism (sHT) by elevated serum TSH in the face of normal thyroid hormones. The high frequency of hypothyroidism among the general population in Western Countries made levothyroxine (LT4) one of the 10 most prescribed drugs. However, circulating TSH has been demonstrated to increase with aging, regardless the existence of an actual thyroid disease. Thus, when confronting an increase in circulating TSH levels in the elderly, especially in the oldest old, it is important to carry an appropriate diagnostic path, comprehensive of clinical picture as well as laboratory and imaging techniques. In the current review, we summarize the recommendations for a correct diagnostic workup and therapeutic approach to older people with elevated TSH value, with special attention to the presence of frailty, comorbidities, and poly-therapy. The treatment of choice for hypothyroid patients is hormone replacement with LT_4 but, it is important to consider multiple factors before commencing the therapy, from the age dependent TSH increase to the presence of an actual thyroid disease and comorbidities. When treatment is necessary, a tailored therapy should be chosen, considering poly-pharmacy and frailty. A careful follow-up and treatment re-assessment should be always considered to avoid the risk of over-treatment. It is important to stress the need of educating the patient for a correct administration of LT_4, particularly when poly-therapy is in place, and the importance of a tailored therapeutic approach and follow-up, to avoid overtreatment.

Keywords: hypothyroidism, elderly, treatment, L-thyroxin, frailty

MODIFICATION OF THE HYPOTHALAMUS-PITUITARY-THYROID AXIS IN THE OLDER PEOPLE

In order to understand the modifications of thyroid axis, from hypothalamus to peripheral tissues, commonly observed during aging, it is noteworthy to briefly review the feed-back mechanisms that rules hormone secretion in young adults. Thyroid hormones are under the controls of TSH levels making the latter a sensitive marker of thyroid function. In this regard, circulating TSH levels in healthy subjects vary according to the circadian rhythm and respond with logarithmically variations to minor changes in serum FT_4 and FT_3 values (1). Thus, the occurrence of abnormal serum TSH in young adults may imply that serum FT_4 and FT_3 are not normal for that person (2). Accordingly, increased serum TSH values indicate a reduced thyroid function while lower TSH levels may underline a hyper-function of the thyroid gland (3). Apart from specific thyroid diseases that may involve older people, the aging process *per se* plays a peculiar role on thyroid axis, from hypothalamus to peripheral thyroid hormone metabolism and action (4–6). The aging process leads to reduced iodine absorption and organification with an altered thyroid response to TSH. Moreover, changing in the TSH bioactivity, in the thyrocyte sensitivity to TSH, in thyroid hormone metabolism as well as in the receptors and co-factors modulating the response to T_3 input has been described (7). Overall, these processes result in reduced thyroid hormone production (8–10). Interestingly, individuals older than 80–85 years presented a nocturnal surge of TSH partially or completely lost with attenuated inhibitory effect of corticosteroids thus, indicating an age depended hypothalamus impairment (2, 11, 12).

A more complex relationship between TSH levels and the aging process has been described in several observation studies even while excluding patients with thyroid disease or autoimmunity. In fact, some experiences (generally case-control) showed a trend toward lower TSH circulating levels in individuals older than 75–80 years and centenarians (4, 13), while more recent cohort studies demonstrated an opposite TSH level behavior during age with a shift toward higher values in older people. In particular, in subjects above 80 years of age, the upper limit of the 95% interval of confidence is around 6.0 mIU/L, reaching 8.0 mIU/L in over-90 s (14–16). Some authors interpreted the reduced TSH levels in centenarians as a central reset of thyroid function in order to prevent an excessive catabolism favoring "physiological aging" (4, 17). It is noteworthy to differentiate this possible physiologic condition from that observed in acute patients and/or in starvation where TSH and T_3 levels are reduced while reverse-T_3 (rT_3) is increased and a poor prognosis *quoad vitam* and *quoad valetudinem* has been described (6, 18). In general, we could hypothesize that the aging process acts for an individual as it does a hypothyroid status resulting in a reduction of the basal metabolism (19). However, to date, on the basis of previous experiences, it is impossible to state if the described reset of hypothalamus-pituitary-thyroid cross-talk in the elderly (due to either reduced TSH secretion or thyroid hormones production) is an effect of the reduced metabolic status or a protective cause preventing the extreme catabolism that characterizes the aging process (19). In addition, when analyzing the aging process on thyroid gland we should mention that the prevalence of specific thyroid diseases increases with age (20) and subclinical thyroid dysfunctions are more frequent than overt diseases (7, 21). Consistently, the prevalence of subclinical hypothyroidism and the presence of autoimmunity against thyroid cells increases with aging (20), thus underling a possible immune mechanism age related that explain this finding.

Some experiences showed that the modifications of pituitary-thyroid axis during aging may have an impact on longevity (7) even if we should report that the most important results on thyroid hormones and lifespan regulation, were obtained in the studies carried out in centenarians (and almost centenarians) (20). In this regard, Atzmon et al. reported that disease-free population of Ashkenazi Jews were characterized by extreme longevity. In details, they have observed higher serum TSH level in centenarians as compared to the control group (younger unrelated Ashkenazi Jews) and also to another control group from The National Health and Nutrition Examination Survey (NHANES). Furthermore, the authors documented an inverse correlation between FT_4 and TSH levels in centenarians and Ashkenazi controls. Another experience in this setting showed a possible thyroid genetic background associated to extreme longevity (22). In particular, two single nucleotide polymorphisms (SNPs) in TSH receptor (TSHR) gene (rs10149689 and rs12050077) correlated with increased TSH level in the Ashkenazi Jewish centenarians and their offspring (22). In line with this, a North Europe study (Leiden Longevity Study) confirmed the role of thyroid genetic background on lifespan regulation. Indeed, the offspring of nonagenarian population presented a low thyroid activity (reduced FT_3 values) and a better metabolic profile compared to their partners with less long-lived parents (8).

Consistently, Corsonello et al. (23) demonstrated an inverse relationship between age and free thyroid hormones independently from TSH levels in a population of Southern Italy (23). Moreover, the offspring of oldest old people presented lower free triiodothyronine (FT_3), FT_4 and TSH levels when compared with age-matched controls (23). Another interesting finding related to the aging process and thyroid function were reported by Gussekloo et al. (24) who firstly showed in a cohort study that oldest old individuals with abnormally high levels of thyrotropin may have a prolonged life span (24). Interestingly, in animal models low levels of T_4 were associated with extended longevity (25–29). For example, a very severe hypothyroidism leading to reduced core body temperature, substantially contributed to remarkable longevity in rodents (25).

A recent report from the Rotterdam study, including over 9,000 healthy home-dwelling subjects, does not confirm the increasing trend of TSH during age, showing instead a progressive reduction of mean serum TSH with a concomitant rising of anti-thyroid peroxidase autoantibody (TPOAb) values with increasing age (30) while the same group provided intriguing results on the peripheral FT_4 values and outcome in the same cohort (31). Those with higher FT_4 values at baseline presented a worse prognosis in term of frailty index (31). Consistently, other experiences in the elderly showed the importance of thyroid hormones peripheral values in term of clinical outcomes (32) reinforcing the hypothesis that, apart from TSH level in very old population, the peripheral pattern of FT_4 and FT_3 may also play a central role in the lifespan regulation at least in older population at risk of frailty (32).

EPIDEMIOLOGY AND CLINICAL EFFECT OF OVERT AND SUBCLINICAL HYPOTHYROIDISM

Over the last decades, the demographic growth in the Occidental Countries determined an increase of the population over 65 years of age. In Italy, which is second only to Japan in the elderly population, the over 80 s are the 6.7% of the overall population, while 22% is constituted by >65 (33) Together with aging, the incidence of chronic diseases increase; thyroid disturbances are frequent among the elderly. Hypothyroidism is defined by an increased level of thyroid stimulating hormone (TSH), with reduced circulating levels of free triiodothyronine (FT_3) and free thyroxine (FT_4) while, subclinical hypothyroidism (sHT) by increased TSH values in the face of normal circulating FT_3 and FT_4 levels (13, 20).

Among the general population in Europe, the prevalence of hypothyroidism varies between 0.2 and 5.3%, while in the USA between 0.3 and 3.7%, this variation probably being due to different iodine intake in diverse areas (34). Many factors may affect the response to excess iodine, among them route and duration of intake, iodine bioavailability and the individual physiopathological status including age, previous iodine intake and thyroid health. Indeed, excess iodine may more likely induce thyroid dysfunction (mainly hypothyroidism) in older subjects with underlying thyroid disease and insufficient iodine intake, than in those who live in iodine-sufficient areas without thyroid disease.

According to the National Health and Nutrition Survey (NHANES III), the global prevalence of hypothyroidism is 4.6%, respectively 0.3% for the overt and 4.3% for the subclinical type resulting the most frequent endocrine disease in the elderly, with a greater prevalence for the female gender (11). In UK, the prevalence of hypothyroidism is around 3.5–5% (35). The prevalence of sHT is variable, depending on the cohort considered (20) and going from 7.5%, as shown in the Wickham study (35) to around 21% in women and 16% in men as shown in the Colorado study (36). As demonstrated by the NANHES III study, TSH circulating levels and anti-thyroid autoantibodies increase with aging; in this study, 14% of the population 85 years old or above had TSH levels higher than 4.5 mUI/L, especially in the female gender (11). In a British population of

6,000 subjects older than 65 years, the prevalence of hypothyroidism was 2%, while the prevalence of sHT was around 2.9%, lower than what found in literature (37). In the same geographic area, the prevalence of sHT in subjects older than 60 years was around 11.6% in females and 2.9% in males (38). The huge difference in the data for the same area after 10 years was theorized to be due to an improved screening campaign and education, together with earlier treatment (39). That explanation may be reasonable, especially considering that the Medicine Utilization Center demonstrated that LT_4 is in the 10 more prescribed drugs in Italy, consistently with the worldwide projections (40).

Considering that hypothyroidism is associated with increased mortality as well as increased incidence of cardiovascular events and cognitive and functional decline, replacement therapy with LT_4 is recommended (41). A population-based retrospective study evaluating more than 2,000 hypothyroid subjects older than 65 years was recently published; the results showed that such condition was independently associated with higher risk of all-causes mortality. In older population, LT_4 replacement therapy was associated instead with a lower risk of mortality. The mortality rate for CVD was similar between the groups receiving or not receiving LT_4 (41). The association between hypothyroidism and all-causes mortality found in that study was in line with a previous longitudinal study, in which the same association in older subjects was found (42), but inconsistent with other epidemiological studies (43–45). Thus, further large prospective, randomized controlled trials (RCT) are necessary to better evaluate the effect of hypothyroidism and LT_4 replacement on cardiovascular and all-causes mortality in the elderly.

Caution needs to be taken, however, in case of subclinical hypothyroidism, in the diagnostic and therapeutic management, particularly in the oldest old (20). Large set of data are available in literature, from meta-analysis and trials, about sHT (46, 47) which, together with the 2013 ETA (European Thyroid Association) guideline for the management of subclinical hypothyroidism, splits the population into two groups, depending on the values of circulating TSH levels, between 4 and 10 mIU/L or above 10 mIU/L (48). Bearing in mind that the thyroid function changes with aging and TSH values tend to increase, it is important to differentiate the age-related modification from the actual gland dysfunction. The most frequent pathogenic mechanism of sHT in the elderly is Hashimoto's thyroiditis (3, 49), although other secondary causes, such as insufficient replacement therapy following surgical or medical procedures (i.e., thyroidectomy or radioiodine treatment) need to be always considered. Hashimoto's thyroiditis, in 90% of cases, has a positive titer of anti-thyroid antibodies [anti-thyroglobulin and/or anti-thyroid peroxidase autoantibodies (TgAb and TPOAb, respectively)]; nonetheless, thyroid tissue damage is supposed to be caused by CD8+ T-lymphocytes, rather than the auto-antibodies themselves (50). Positive anti-thyroid autoantibody titers may represent a useful information not only about the presence of autoimmune thyroiditis, but also about the chance of progression to overt hypothyroidism, which has an yearly incidence of 4.3% in TPOAb positive patients, compared to 2.6% in the negative ones (50, 51). When demonstrated, the monitoring of the titer of anti-thyroid antibodies doesn't add much information, since it varies with the TSH levels (52). In the NHANES III study, the cohort of 13,000 healthy subjects was regularly followed up; the repeated dosage of FT_3, FT_4, TSH, TgAb, and TPOAb, showed that 10% of the subjects were positive for TgAb and 11% for TPOAb (11). In the around 20% of cases of antibody-negative sHT individuals, the diagnosis would be supported by the presence of tissue inhomogeneity and hypo-echogenicity at the thyroid US scan (53). Another possible cause of hypothyroidism in the elderly is iatrogenic. Drugs interfering with L-thyroxin absorption, as well as drugs potentially damaging the gland tissue such as ß-blockers, interferon-α, interleukin-2, lithium, ethionamide, tyrosin-kinase inhibitors, and thyrostatic medications (methimazole, perchlorate, and propylthiouracil), could determine hypothyroidism. The drug-induced damage is usually transient, and a periodical monitoring of the gland function, at least twice a year, is recommended (49). It has been widely accepted that thyroid hormones play a role in the cardiovascular system, modulating the adrenergic system activity, regulating the vascular peripheral resistance and in the protein synthesis (7). Unfortunately, while the impact of sHT on the cardiovascular (CV) system among the young adult has been recognized, among the elderly is still a matter of debate (20, 41, 54) especially since no RCTs have been conducted so far evaluating the impact of LT4 therapy on CV outcomes. A recent study involving over 2,100 subjects longitudinally, aimed at identifying a possible relationship between sHT and metabolic syndrome in the elderly. In the population examined, TSH level above 10 mUI/L was associated with higher odds of prevalent metabolic syndrome (21); circulating TSH levels above 10 mUI/L have been demonstrated to increase the risk of heart failure (HF) as well (7). The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) showed an association between HF and sHT over a follow up period of 3.2 years, in a population of 70–82 years old subjects, for TSH circulating levels above 10 mUI/L, while no association was found below that threshold (54). A large meta-analysis confirmed the association between HF and TSH levels above 10 mUI/L (or below 0.10 mUI/L) (46). More conflicting results has been reported for the relationship between sHT and coronary heart disease (CHD), more consistent in the younger population (7) (55), although a large meta-analysis showed an increased risk of CHD events and mortality for TSH levels above 10 mUI/L, across 35 years follow up, also adjusting for sex and age (45).

Thyroid hormones play a role in few metabolic functions, such as thermo regulation, oxygen consumption, glucose uptake, contra-insular activity, cholesterol mobilization and low-density lipoprotein (LDL) receptors expression in the liver (56). It is common, in hypothyroidism, to find increased levels of cholesterol and its sub-fractions (57); part of that is related to reduced cholesterol clearance, due to a reduced expression of the LDL receptor gene. Increased level of triglycerides is also a common finding in overt hypothyroidism, generally unmodified in sHT, following a reduced lipogenesis and lipase activities (58). The role of thyroid homeostasis in the cognitive development is widely known and accepted; not completely clear is the effect of thyroid failure, overt or mild, in the elderly and the impact it may have on cognitive impairment (59). An increased risk of Alzheimer's disease development has been seen in women at the lowest (< 1.0 mIU/L) and highest (>2.1 mIU/L) tertiles of serum TSH concentration in the Framingham study (60). Other studies showed interesting results; in the Health, Aging and Body Composition study, the risk of developing dementia was higher in subclinical hyperthyroidism, but not in sHT subjects (61), results consistent with a previous meta-analysis of Rieben et al. (62). Another recent meta-analysis showed a significant relationship between higher levels of circulating TSH and impaired cognitive performance in younger population (< 75 years of age) (63). On the other hand, a longitudinal study conducted on a cohort of cognitively normal subjects aged 60–90 years didn't find any relationship between TSH and thyroid hormones and hippocampal atrophy or risk of developing dementia (64). The inconsistent results available despite the important role played by thyroid function raise the need of long-term longitudinal studies, involving elder population, including the oldest old.

HYPOTHYROIDISM IN THE ELDERLY: WHEN TO TREAT?

Consistently to the principle that the therapy of choice for glandular deficiency is the replacement therapy, for overt hypothyroidism the first choice is LT_4 replacement, also in older patients (65). The appropriate treatment of hypothyroidism, dealing to the resolution of the disease, leads to the release of symptoms, such as fatigue, constipation, increased sensitivity to cold, muscle weakness, and increased weight; improvement has been demonstrated in cognitive executive and cardiovascular functions (66). When considering the treatment of sHT in the elderly (especially in those older than 75–80 years), the approach has to be more cautious. It has been demonstrated in several studies that LT_4 replacement therapy should be started when the TSH values are above 10 mUI/L, this being considered the value above which the risk of health disorders rises (7, 21, 46, 54). However, it is important to keep an approach on a case-by-case basis; this is particularly important in patients with potential other cardiovascular risk factors, which could hide the symptoms and signs related to sHT, already potentially less evident. Among the older population, it is also important to evaluate the potential frailty and comorbidities (66), appropriately tailoring the therapy. On that note, the evaluation of TSH levels and the trend over time is crucial; the international guidelines have set the cut-off level to 10 mUI/L, double checked and confirmed over 3 and 6 months before commencing the treatment (48, 49). Other than the TSH levels, the clinician should check the clinical presentation with signs and symptoms before deciding for any therapeutic approach (48), bearing in mind that many symptoms are unspecific (i.e., fatigue, constipation, sleeping pattern alteration, and fatigue), especially in the elderly with comorbidities (32). A well-structured approach, including a multidimensional geriatric assessment (67), comprehends a wide evaluation, which includes laboratory tests (FT_3, FT_4, TgAb, TPOAb) and US scan, to identify potential causes of thyroid failure (gland atrophy or autoimmune thyroiditis), responsible for permanently increased TSH levels. Whilst TSH levels tend to increase with aging, usually they don't exceed 7–8 mIU/L (14). In addition to the laboratory and imaging evaluation, the collection of a well accurate pharmacological history for drugs potentially affecting the thyroid function, such as amiodarone, lithium etc., is very important.

In 2017, Stott et al. conducted a double blinded, randomized, placebo-controlled study aiming to evaluate the efficacy of the therapy with LT_4 on a large cohort (737 subjects) of older patient (mean age 74.4 years) with persistent sHT (mean entry TSH level: 6.40 ± 2.01 mIU/L) (68). The primary outcomes of the study were the changes, in 1 year, in the Hypothyroid Symptoms score and Tiredness score on a thyroid-related quality-of-life questionnaire. At the follow up evaluation, at 1 year, mean serum TSH level in the treatment group was 3.63 ± 2.11 and 5.48 ± 2.48 mIU/L in the placebo group. Among the groups, there were no differences in the quality of life measured with the questionnaire, nor difference in the adverse events of interest. The study concluded that the treatment with LT_4 failed to provide an actual benefit in sHT subjects. However, some limitations in the study should be taken into account: serum TSH level at baseline was above 10 mUI/L only in few subjects, symptoms' level was low, and the presence of autoimmunity was not assessed. In particular, the latter limitation needs to be considered, since autoantibody positive patients are more likely to have progressive hypothyroidism, therefore long-term treatment could be actually beneficial (68). The study, moreover, was underpowered to detect the incidence of the LT_4 therapy on cardiovascular events or mortality. Larger studies with a large cohort of older subjects with actual thyroid disease (i.e., with positive Ab titers) are not available at the moment. Our recommendation, in the elderly with sHT, is to approach the clinical management not only considering serum TSH levels and the 10 mUI/L cut-off, but also evaluating the presence of autoimmune thyroiditis as well comorbidities (especially HF) (69). On that note, the evaluation of the presence of frailty is crucial, considering how much impact it could have on the patient's quality of life and the clinical prognosis (32, 70): frail subjects are more likely to be affected by drugs side effect, and the risk of overtreatment, or poor compliance needs to be accounted in the clinical workup. The suggested clinical management of sHT in either fit or frail older patients is summarized in Figures 1, 2. In case of fit older (65–75 years) patients, LT_4 replacement should be commenced when TSH levels are above 10 mUI/L (48, 49) while, fit oldest old (>75–80 years) should be treated when clear signs and symptoms of thyroid disease are present, after careful evaluation of cardiovascular and cognitive comorbidities; in absence of that, the strategy of choice should be the observation over time, in agreement with the ETA 2013 guidelines (48). A more cautious approach is suggested in frail elderly subjects, as shown in Figure 2. In frail subjects with TSH levels above 10 mUI/L, the wait-and-see strategy should be the one of choice, treating subjects in the 65–75 years of age range in presence of actual thyroid disease, symptoms of hypothyroidism and/or comorbidities potentially worsened by mild thyroid failure (i.e., heart failure). In case of serum TSH levels between 6 and 10 mUI/L, LT_4 replacement therapy should be considered in “fit” subjects with risks factors for thyroid disease progression, such as anti-thyroid Ab, US pattern suggestive of disease, female gender; in absence of thyroid disease progression risk factors, an observation period with follow up of thyroid function every 3–6 months is suggested, commencing the therapy if the TSH level increases above 10 mIU/L (Figure 1). In the same range of values, but in frail subjects (Figure 2), the observation strategy is the one of choice. In frail patients younger than 75 years, with TSH levels below 10 mIU/L, the strategy of choice is to avoid LT_4

replacement, unless the TSH level would progressively increase above 10 mIU/L during follow up, in presence of comorbidities potentially negatively influenced by mild thyroid failure, or in case of positive anti-thyroid auto antibody titers. In case of "fit" elderly younger than 75 years, with positive anti-thyroid autoantibody titer, symptoms of hypothyroidism and/or comorbidities influenced by mild thyroid failure, a trial with LT4 replacement should be considered (Figure 1). For all the subjects receiving replacement therapy, the titration of LT_4 should be done from around 0.3–0.4 µg/Kg/day with increments by 10–15% after 6–8 weeks, if necessary, considering the optimal target values between 2.5 and 3.5 mIU/L, in agreement with international guidelines (48, 49). The regular monitoring and follow up of thyroid function is recommended over time, especially in the oldest old, to avoid over-treatment, which is known to negatively impact on cardiovascular an osteo-muscolar systems (48, 49).

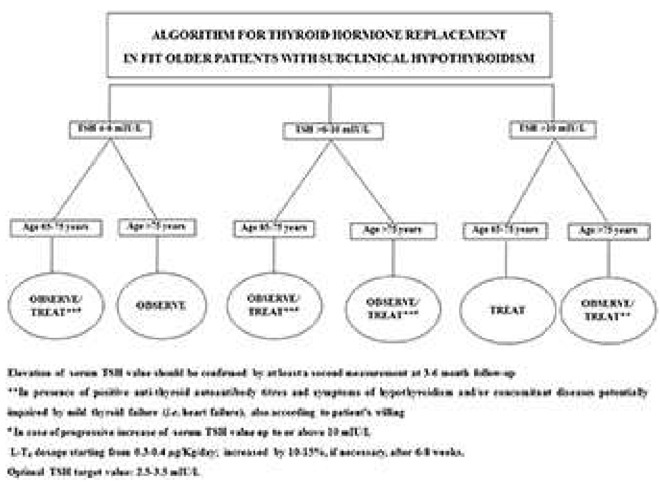

FIGURE 1. Suggested strategy of care according to either serum TSH value or the clinical features in fit older patients.

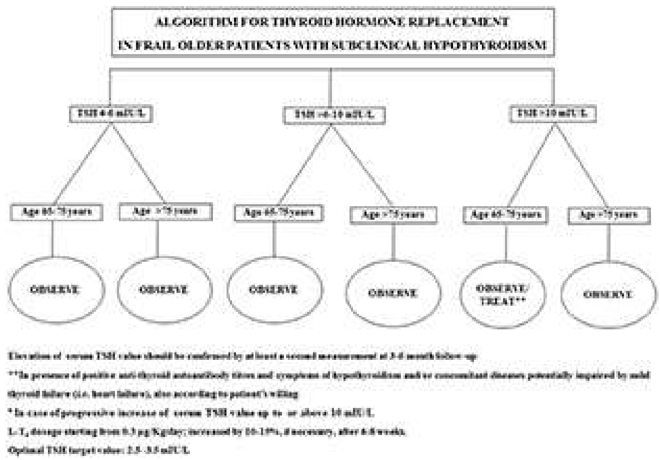

FIGURE 2. Suggested strategy of care according to either serum TSH value or the clinical features in frail older patients.

Different formulations are available for the replacement therapy; the most used is the LT_4 tablet, which is usually the first choice in absence of swallowing problems. However, considering the delicate process of absorption of the LT_4, which can be influenced by several gastro-intestinal factors (71), some "rules" in regards of food and concomitant drugs administration should be followed (48, 49).

A debate is still open regarding the use of combined therapy T_4+T_3. Few studies have been conducted; most of the studies evaluated in a recent review of the literature failed to demonstrate a clear advantage with the combined therapy (72). The same lack of advantage over the monotherapy has been seen also in two different meta-analyses (73, 74). A large study evaluating the outcomes over 17 years follow up in population undertaking T_3, mostly associated with T_4, compared to a population receiving only LT_4, didn't show any difference in the cardiovascular events, atrial fibrillation, fractures, diabetes mellitus or death; the group receiving T_3 showed an increased rate of use of antipsychotic drugs (75). According to the 2012 ETA guidelines (10), the combined therapy T_4/T_3 should be used only in case of persistent complaint from the patient despite normal values of TSH with the monotherapy, after adequate education regarding the chronicity of the thyroid condition. Moreover, the combined therapy should be interrupted if the clinical improvement is not reached within 3 months (76). Thus, taking in mind the potential drawbacks of T_3 therapy, the combined treatment with T_4/T_3 is generally not advised in older hypothyroid patients, especially in those older than 75 years.

Considering how variable the thyroid hormones could be among the general population, due to the influence of genetic, demographic (i.e., age and gender) and environmental factors, it is important to tailor and personalize the individual's treatment and follow up approach.

CONCLUSIONS

Hypothyroidism, overt or subclinical, is a very frequent chronic disease among the older population; however, TSH circulating levels have been demonstrated to increase with aging, regardless the existence of an actual thyroid disease. For this reason, when confronting an increase in TSH circulating level in a patient older than 65 years of age, and even more carefully in the oldest old, it is important to carry an appropriate diagnostic path, comprehensive of clinical picture, laboratory tests, in particular checking for anti-thyroid autoantibodies, and US scan. Moreover, in the older population, the presence of frailty needs to be considered and addressed (77). The therapy of choice is hormone replacement with LT_4, whichever pharmacologic form is more adequate, starting with a dosage of 0.3–0.4 μg/Kg/day and titrating by 10–15% after 6–8 weeks, aiming to keep an optimal TSH level of 2.5–3.5 mIU/L. It is important to stress the need of educating the patient for a correct administration of the therapy, particularly when poly-therapy is in place and the importance of a tailored therapeutic approach and follow up, to avoid overtreatment.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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The Differential Effect of Excess Aldosterone on Skeletal Muscle Mass by Sex

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The effects of excess aldosterone on skeletal muscle in individuals with primary aldosteronism (PA) are unknown. To examine the effects of aldosterone on skeletal muscle mass in patients with PA, by sex, 309 consecutive patients were enrolled. Skeletal muscle and fat mass of 62 patients with PA were compared with those of 247 controls with non-functioning adrenal incidentaloma (NFAI). Body composition parameters were measured using bioelectrical impedance analysis, and plasma aldosterone concentration (PAC) was measured using radioimmunoassay. The PAC in all women, but not in men, showed an inverse association with both appendicular skeletal muscle mass (ASM) ($\beta = -0.197$, $P = 0.016$) and height-adjusted ASM (HA-ASM) ($\beta = -0.207$, $P = 0.009$). HA-ASM in women (but not in men) with PA was 5.0% lower than that in women with NFAI ($P = 0.036$). Furthermore, women with PA had a lower HA-ASM than 1:1 age- and sex-matched controls with NFAI by 5.7% ($P = 0.049$) and tended to have a lower HA-ASM than 1:3 age-, sex-, and menopausal status-matched controls without adrenal incidentaloma (AI) by 7.3% ($P = 0.053$). The odds ratio (OR), per quartile increase in PAC, of low HA-ASM in women was 1.18 [95% confidence interval (CI), 1.01–1.39; $P = 0.035$]. The odds of HA-ASM in subjects with PA were 10.63-fold (95% CI: 0.83–135.50) higher, with marginal significance ($P = 0.069$) than in those with NFAI. Skeletal muscle mass in women with PA was lower than that in women with NFAI; suggesting that excess aldosterone has adverse effects on skeletal muscle metabolism.

Keywords: primary aldosteronism, aldosterone, skeletal muscle mass, sarcopenia, sex

INTRODUCTION

Aging is associated with sarcopenia, which is characterized by loss of skeletal muscle mass and strength, and/or decline in physical performance (1). Previous studies confirm an association between sarcopenia and adverse health outcomes such as impaired cardiopulmonary performance, reduced physical capability, and increased disability and mortality (2). Asia, including Korea, is a region with a rapidly aging population; thus, sarcopenia is increasingly prevalent (3). Indeed, a national survey in Korea revealed that 11.9% of women and 12.1% of men have sarcopenia (4); therefore, it is becoming a major challenge in terms of healthy aging.

Evidence suggests that inhibiting the renin–angiotensin–aldosterone system (RAAS) may prevent the development of sarcopenia (5, 6). Indeed, the treatment of older people without congestive heart failure (CHF) with angiotensin I converting enzyme (ACE) inhibitors improves physical performance (6, 7). Aldosterone, a mineralocorticoid, is a terminal hormone of the RAAS; therefore, it may have deleterious effects on skeletal muscle (5). Aldosterone increases the loss of magnesium via the urine, thereby depleting the levels of magnesium in the muscle where it is essential for activating the Na^+/K^+ pumps, which regulates muscle contraction (8, 9). Aldosterone also suppresses insulin-mediated glucose uptake and increases oxidative stress in skeletal muscle (10). Furthermore, plasma aldosterone concentration (PAC) in CHF patients with cardiac cachexia is higher than that in age-matched controls without CHF (11). Blocking the mineralocorticoid receptor (MR) for aldosterone with spironolactone prevents the loss of skeletal myocytes (12), improves vascular endothelial function and muscle blood flow (13), and improves muscle contractile performance by increasing the magnesium levels and up-regulating Na^+/K^+ pumps (8). To date, the majority of studies examining the detrimental effects of aldosterone on skeletal muscle have been conducted in animals, in patients with CHF, or patients with alcoholic liver cirrhosis (LC), in whom muscle wasting may be caused by cachexia with impaired cardiac function or the toxic effects of alcohol. Although cachexia and sarcopenia show common pathophysiological mechanisms of underlying muscle dysfunction and muscle loss (14), there are several differences between them. Cachexia is associated with major diseases such as infections, cancer, heart disease, chronic kidney disease, chronic obstructive pulmonary disease, and stroke (15). Cachexia is weight loss caused not only by inflammatory cytokines but also by proteolytic inducers, derived from underlying diseases. On the contrary, sarcopenia is the loss of muscle mass and function, mainly associated with aging. Sarcopenia is caused by failure of satellite cell activation or by the promotion of proinflammatory cytokines (16). Therefore, it is unclear whether excess aldosterone contributes to the development of sarcopenia in the general population.

Primary aldosteronism (PA) is a disease of the adrenal gland and is characterized by levels of aldosterone that are inappropriately high for sodium status (17). Therefore, PA is a good model in which to examine the effects of excess aldosterone on human skeletal muscle. To the best of our knowledge, no study has examined skeletal muscle mass in individuals with PA. Therefore, we examined the association between PAC and skeletal muscle mass and compared the body composition of Korean patients with PA with that of those with non-functioning adrenal incidentaloma (NFAI).

MATERIALS AND METHODS

Study Participants and Protocol

Consecutive patients (*n* = 919) with adrenal incidentaloma (AI), newly diagnosed at Asan Medical Center (AMC; Seoul, Korea) between July 2011 and December 2015, were screened (Supplementary Figure 1). Diagnosis of AI was based on the detection of an adrenal mass (size ≥1 cm) using computed tomography (CT), which was performed as part of an investigation for an unrelated disease. All patients with AI underwent biochemical evaluation to test for hormonal abnormalities. Of these, 597 patients were referred from the Health Promotion Center due to AI where they underwent bioelectrical impedance analysis (BIA); therefore, they were eligible for inclusion in this study. Two hundred and thirty eight patients with suspected hypercortisolism, pheochromocytoma, adrenal metastasis, adrenal carcinoma, adrenal tuberculosis, congenital adrenal hyperplasia, or pseudo-Cushing's syndrome were excluded. In addition, 80 patients who had taken estrogen, steroids, or thyroid hormone, or had a disorder (such as hyperthyroidism) that might affect muscle mass, were excluded. Before measuring the PAC (ng/dL) and plasma renin activity (PRA, ng/mL/h) to detect possible case of PA [determined by calculating the aldosterone to renin ratio (ARR)], all antihypertensive medications, such as angiotensin II receptor blockers ACE inhibitors, were withdrawn for ≥4 weeks to prevent possible interference with the results (17). If absolutely necessary, subjects received α-adrenergic blocker (e.g., doxazosin) and/or a non-dihydropyridine slow-release antagonist calcium channel blocker (e.g., verapamil) in accordance with recent guidelines (17). All patients were encouraged to continue with oral potassium supplementation in case of hypokalemia. And there were no restrictions on the consumption of dietary salt before testing. The subjects in the matched control group with NFAI were randomly selected from among patients who undertook a screening test via the Health Promotion Center, at AMC (Seoul, Korea) within the same periods as those in the PA group. The 57 controls were matched (1:1) to the cases according to both age (within 2.0 years) and sex.

The screening test result was considered positive if the ARR was ≥30. The diagnosis of PA was confirmed by a non-suppressed PAC value of >10 ng/dL after an intravenous saline infusion test (2 L of 0.9% saline infused over 4 h) (17). PA was excluded if the post-infusion PAC value was <5 ng/dL. The intravenous saline infusion test was repeated if the post-infusion PAC value was 5–10 ng/dL. However, PA was diagnosed without a confirmatory test in those with spontaneous hypokalemia, a PRA below the detection limits, and a PAC >20 ng/dL (17). Finally, 62 patients were diagnosed with PA (29 women and 33 men) and 247 patients were diagnosed with NFAI (76 women and 171 men) (Supplementary Figure 1). The 57 subjects in the control group with NFAI who were matched 1:1 to patients with PA in terms of sex and age (±2.0 years) were randomly selected from the 247 patients with NFAI. Furthermore, 186 controls without AI who were matched 1:3 to patients with PA in terms of sex and age (±1.0 years) were randomly selected from patients who had BIA data, which were performed in the Health Promotion Center.

Height (cm) and weight (kg) were measured (participants wore light clothing without shoes), and body mass index (BMI; kg/m^2) was calculated. Blood pressure (BP, mmHg) was measured twice using a mercury manometer after the patient had rested for >15 min; the average value was recorded. Mean arterial pressure (MAP) was calculated as [systolic BP + (2 × diastolic BP)]/3 (mmHg). The following patient information was obtained from an interview-assisted questionnaire: regular outdoor exercise (≥30 min/d), alcohol intake (≥3 U/d), smoking habits (current smoker), previous medical or surgical procedures, history of medication use, and reproductive status (including menstruation).

The study was approved by the Institutional Review Boards at AMC, and all participants provided written informed consent.

Bioelectrical Impedance Analysis (BIA)

Body composition was measured using a direct segmental multi-frequency BIA (In-Body 720; Biospace Co., Ltd., Seoul, Korea) apparatus. The In-Body 720 automatically estimates the weight, body mass index (BMI, kg/m^2), fat mass (FM, kg), percent fat mass (pFM, %), and skeletal muscle mass in the arms and legs. The pFM is the ratio of FM to total body weight. Lean mass (LM, kg), is the total muscle mass. Appendicular skeletal muscle mass (ASM, kg) was calculated as the summed skeletal muscle mass in the arms and legs. Upper limb ASM (UL-ASM, kg) was calculated as the summed skeletal muscle mass in both arms, and lower limb ASM (LL-ASM, kg) was calculated as the summed skeletal muscle mass in both legs. As suggested by the Consensus Report of the Asian Working Group for Sarcopenia (1), height-adjusted ASM (HA-ASM, kg/m^2) was defined as ASM divided by height in meters squared ($ASM/height^2$)

(1). Low skeletal muscle mass was defined in terms of HA-ASM using a cutoff point of <6.75 kg/m^2 for men and <5.07 kg/m^2 for women.

Measurement of Hormone Levels and Biochemical Parameters

Morning blood samples were drawn after an overnight fast. The PAC and PRA were measured by radioimmunoassay (SPAC-S aldosterone and PRA kits, respectively; TFB Inc., Tokyo, Japan) using a Cobra II Gamma Counter (Packard Instrument Co., Meriden, CT). For the PAC assay, the lower limit of detection was >1.53 ng/dL, and the intra-assay and inter-assay coefficients of variation (CVs) were <3.2 and <6.7%, respectively. For the PRA assay, the lower limit of detection was >0.09 ng/mL/h and the intra-assay and inter-assay CVs were <8.3 and <9.7%, respectively.

Serum potassium levels were measured using a Roche ISE Standard Low/High (Roche Diagnostics, Mannheim, Germany) ion selective electrode (ISE) and a Cobas 8000 ISE analyzer (Roche Diagnostics). The intra-assay and inter-assay CVs were 0.5 and 1.6%, respectively. Serum creatinine was measured in a kinetic colorimetric assay using the Roche CREAJ2 kit (Roche Diagnostics) and a Cobas c702 module (Roche Diagnostics). The intra-assay and inter-assay CVs were <2.3 and <2.7%, respectively. Glomerular filtration rate (GFR) was estimated using the Cockcroft–Gault equation (18).

Statistical Analysis

Data are expressed as the mean ± standard deviations (SD), the median (interquartile range), or number (percentage) unless stated otherwise. Baseline characteristics were compared using Student's *t*-test or the Mann–Whitney *U*-test (continuous variables) or the χ^2 test (categorical variables). To investigate the correlation of PAC with age, we performed Pearson's correlation analysis in patients with NFAI. Interaction analysis was performed to test whether the association between PAC (presented as a continuous variable) and parameters of body composition was modified by sex (coded as 0 and 1 for women and men, respectively, and expressed as a categorical variable). The association between PAC and ASM, UL-ASM, LL-ASM, HA-ASM, FM, and pFM was evaluated by multiple linear regression analyses after adjusting for confounding factors (age, menopausal status in women, BMI, regular outdoor exercise, alcohol intake, current smoking, MAP, K^+ levels, and GFR). To further analyze the differences in the magnitude of the association between PAC and UL-ASM and LL-ASM, the corresponding regression coefficients were compared using a previously reported equation, which is an extension of the *t*-test with unstandardized β-coefficients and standard error (SE) (19). After women and men were assigned to four groups according to PAC quartile, the multivariable-adjusted least squares mean value (95% CIs) of HA-ASM was calculated with respect to PAC quartile; these were then compared using analysis of covariance (ANCOVA) after adjusting for potential confounding factors. The multivariable-adjusted least squares mean values (95% CIs) for ASM, HA-ASM, UL-ASM, LL-ASM, FM, and pFM based on the absence/presence of PA were calculated and then compared using ANCOVA, after adjusting for potential confounding factors. The multivariable-adjusted least squares mean values (95% CIs) for HA-ASM and LM based on the absence/presence of PA were calculated and then compared with PA cases after adjusting for potential confounding factors, with the 1:1 age- and sex-matched controls with NFAI or 1:3 age-, sex-, and menopausal status-matched controls without AI, using ANCOVA. Multiple logistic regression analyses was performed to calculate the odds ratio (OR) and 95% CIs for an association between low skeletal muscle mass per increase in PAC or the presence of PA (after adjusting for potential confounders). A receiver operating characteristics (ROC) curve was constructed and the area under the curve (AUC) was measured to assess the ability of PAC to predict low skeletal muscle mass. All statistical analyses were performed using SPSS, version 22.0 (SPSS Inc., Chicago, IL). A P value <0.05 was deemed statistically significant.

RESULTS

The 309 participants enrolled in the study were categorized according to the PA status. The baseline characteristics are presented in Table 1. Women with PA (n = 29) tended to be younger than women with NFAI (n = 76; P = 0.061). There was no significant difference in age, menopausal status of women, and in height, weight, and GFR in both sexes, between the PA group and the NFAI group. As expected, both women and men with PA had higher systolic blood pressure (systolic BP), MAP, PAC, and ARR; and lower K^+ levels and PRA values, than women with NFAI. PAC was negatively correlated with age ($\gamma = -0.162$, P = 0.001 for men and $\gamma = -0.209$, P = 0.001 for women) in patients with NFAI (data not shown). The baseline body composition differed by sex. The pFM was significantly higher in women (32.2 ± 6.4%) than men (23.0 ± 5.1, P <0.001). LM was significantly higher in men (53.5 ± 6.3 kg) than women (38.5 ± 3.9, P <0.001). ASM also showed the same tendency as LM (16.1 ± 2.4 kg for women vs. 23.7 ± 3.3 kg for men, P <0.001) (data not shown).

Table 1. Baseline characteristics of the study participants (n = 309).

Variables	Women (n = 105)			Men (n = 204)		
	NFAI (n = 76)	PA (n = 29)	P	NFAI (n = 171)	PA (n = 33)	P
Age (y)	54.6 ± 7.8	57.9 ± 8.3	0.061	55.2 ± 7.8	57.5 ± 6.0	0.106
Postmenopausal, n (%)	59 (77.6%)	24 (82.8%)	0.564	–	–	–
Height (cm)	158.4 ± 5.2	157.6 ± 5.0	0.446	169.7 ± 6.7	170.4 ± 5.1	0.574
Weight (kg)	61.4 ± 9.1	60.5 ± 10.0	0.674	74.7 ± 10.0	77.8 ± 10.8	0.116
BMI (kg/m^2)	25.2 ± 7.1	24.3 ± 3.3	0.535	26.0 ± 2.8	26.9 ± 2.5	0.084
Systolic BP (mmHg)	**123.4 ± 13.6**	**134.0 ± 16.0**	**0.001**	**126.0 ± 11.7**	**142.3 ± 15.5**	**<0.001**
Diastolic BP (mmHg)	76.4 ± 8.6	79.2 ± 10.2	0.167	**79.6 ± 8.6**	**87.1 ± 10.3**	**<0.001**
MAP (mmHg)	**92.1 ± 9.6**	**97.4 ± 10.4**	**0.014**	**95.1 ± 8.9**	**105.5 ± 1.8**	**<0.001**
Current smoker, n (%)	3 (3.9%)	0 (0.0%)	0.278	58 (33.9%)	7 (21.2%)	0.152
Alcohol intake ≥3 U/day, n (%)	3 (5.1%)	0 (0.0%)	0.242	**16 (12.3%)**	**9 (32.1%)**	**0.009**
Regular exercise ≥30 min/day, n (%)	19 (25.0%)	2 (6.9%)	0.054	61 (35.7%)	5 (15.2%)	0.210
GFR (mL/min)	94.2 ± 27.1	92.9 ± 39.2	0.870	95.5 ± 22.0	98.0 ± 17.8	0.536
K^+ (mEq/L)	**4.1 ± 0.3**	**3.9 ± 0.5**	**0.014**	**4.3 ± 0.3**	**4.1 ± 0.4**	**0.005**
PAC (ng/dL)	**13.1 ± 8.6**	**26.0 ± 9.5**	**<0.001**	**11.8 ± 7.0**	**23.7 ± 8.9**	**<0.001**
PRA (ng/mL/h)	**1.1 ± 0.9**	**0.5 ± 0.9**	**0.008**	**2.7 ± 4.2**	**0.3 ± 0.2**	**<0.001**
ARR ([ng/dL]/[ng/mL/h])	**29.0 ± 36.6**	**97.6 ± 61.8**	**<0.001**	**18.6 ± 33.8**	**100.3 ± 68.7**	**<0.001**

Data are expressed as the mean ± standard deviation or as the median (interquartile range), unless indicated otherwise. Bold numbers indicate statistically significant values. NFAI, non-functioning adrenal incidentaloma; PA, primary aldosteronism; BMI, body mass index; BP, blood pressure; MAP, mean arterial pressure; GFR, glomerular filtration rate; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, aldosterone to renin ratio.

Next, we tested whether any relationship existing between PAC and body composition is affected by sex. The results showed sex effects for ASM, UL-ASM, LL-ASM, and HA-ASM (P value for interaction = 0.008–0.030). Therefore, data for men and women were analyzed separately.

The results of the multiple linear regression analyses performed to identify any independent associations between PAC and ASM, UL-ASM, LL-ASM, HA-ASM, FM, and pFM are presented in Table 2. For women, a higher PAC was significantly associated with lower ASM, UL-ASM, LL-ASM, and HA-ASM, but not with FM and pFM, after adjusting for confounders. Despite a lack of statistical significance (P = 0.201), the magnitude of the inverse association between PAC and LL-ASM (β = −0.032) was larger than that between PAC and UL-ASM (β = −0.013). There was no statistically significant association between PAC and ASM, UL-ASM, LL-ASM, HA-ASM, FM, and pFM in men. Furthermore, there was no statistically significant association between PRA or ARR and ASM, UL-ASM, LL-ASM, and HA-ASM in men or women (data not shown).

Table 2. Multiple linear regression analysis of the association between plasma aldosterone concentration (PAC) and ASM, UL-ASM, LL-ASM, HA-ASM, FM, and pFM (n = 309).

Variable	Women (n = 105)				Men (n = 204)			
	β	SE	*β*	*P*	β	SE	*β*	*P*
ASM (kg)	**−0.045**	**0.018**	**−0.197**	**0.016**	0.028	0.029	0.070	0.343
UL-ASM (kg)	**−0.013**	**0.005**	**−0.197**	**0.012**	0.006	0.008	0.052	0.431
LL-ASM (kg)	**−0.032**	**0.014**	**−0.189**	**0.025**	0.022	0.022	0.074	0.338
HA-ASM (kg/m^2)	**−0.015**	**0.006**	**−0.207**	**0.009**	−0.006	0.006	−0.060	0.341
FM (kg)	−0.047	0.026	−0.084	0.077	−0.011	0.031	−0.017	0.717
pFM (%)	−0.012	0.044	−0.022	0.783	−0.035	0.039	−0.058	0.371

P values were calculated by multiple linear regression analysis of the PAC, adjusted for age, menopausal status (in women), body mass index, regular outdoor exercise, alcohol intake, current smoking, mean arterial pressure, glomerular filtration rate, and K^+ level. Significant results (P < 0.05) are shown in bold.

ASM, appendicular skeletal muscle mass; UL-ASM, upper limb ASM; LL-ASM, lower limb ASM; HA-ASM, height-adjusted ASM; FM, fat mass; pFM, percent FM; β, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient;

HA-ASM: height-adjusted ASM [HA-ASM (kg/m^2)], defined as ASM divided by body height in meters squared (ASM/height^2).

After adjusting for potential confounders, estimation of the multivariable-adjusted least squares mean HA-ASM according to PAC quartiles (Figure 1) revealed that women in the highest quartile (quartile 4: 23.6–51.0 ng/dL) had a lower HA-ASM than those in the other quartiles (quartiles 1–3: 1.2–23.5 ng/dL); specifically, the values were 7.8% lower than those in quartile 1 (*P* = 0.007), 7.9% lower than those in quartile 2 (*P* = 0.005), and 9.0% lower than those in quartile 3 (*P* = 0.002) (Figure 1A). There was no significant difference in HA-ASM between PAC quartiles 1, 2, and 3 (*P* = 0.660–0.974). For men, the association between PAC and HA-ASM did not show a threshold effect (Figure 1B).

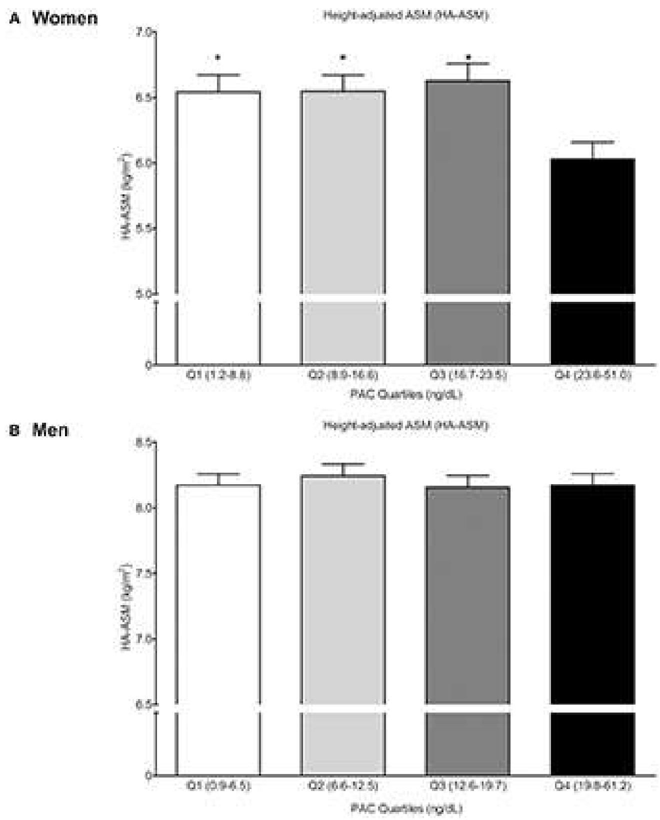

FIGURE 1. Height-adjusted ASM (HA-ASM) according to plasma aldosterone concentration (PAC) quartile. **(A)** is for women and **(B)** is for men. Values represent estimated means, with 95% confidence intervals calculated from the analysis of covariance (ANCOVA) after adjusting for age, menopausal status in women, body mass index, regular outdoor exercise, alcohol intake, current smoking, mean arterial pressure, glomerular filtration rate, and K^+ levels. *Significantly difference occurred with the highest quartile (Q4) (ANCOVA with *post-hoc* analysis).

Next, we used ANCOVA to estimate differences in ASM, HA-ASM, UL-ASM, LL-ASM, FM, and pFM between participants with PA and NFAI (after adjusting for all potential confounders) (Figure 2). For women with PA, LL-ASM was 5.4% lower (P = 0.046) and HA-ASM was 4.9% lower (P = 0.036) than those for women without PA (Figure 2A). There was no difference in UL-ASM. For men, there was no statistically significant difference in ASM, HA-ASM, UL-ASM, LL-ASM, FM, and pFM between the PA and NFAI groups (Figure 2B). We compared HA-ASM and LM between patients with PA (n = 57) and 1:1 age- (±2.0 years), and sex- matched controls with NFAI (n = 57) (Supplementary Table 1, Supplementary Figure 2). As shown in Supplementary Figure 2; 24 women with PA had lower HA-ASM than 1:1 age- and sex-matched 24 women with NFAI controls by 5.7% (P = 0.049) after adjusting for all potential confounders. For men, there was no statistically significant difference in HA-ASM and LM between the PA (n = 33) and 1:1 age-, and sex-matched controls with NFAI (n = 33). We also compared HA-ASM and LM between patients with PA (n = 62) and 1:3 sex-, age- (±1.0 years), and menopausal status-matched controls without AI (n = 186) (Supplementary Table 2, Supplementary Figure 2). Women with PA (n = 29) tended to have lower HA-ASM than 1:3 age-, sex-, and menopausal status-matched controls women without AI (n = 87) by 7.3% (P = 0.054). For men, there was no statistically significant difference in HA-ASM and LM between the PA (n = 33) and control groups (n = 99).

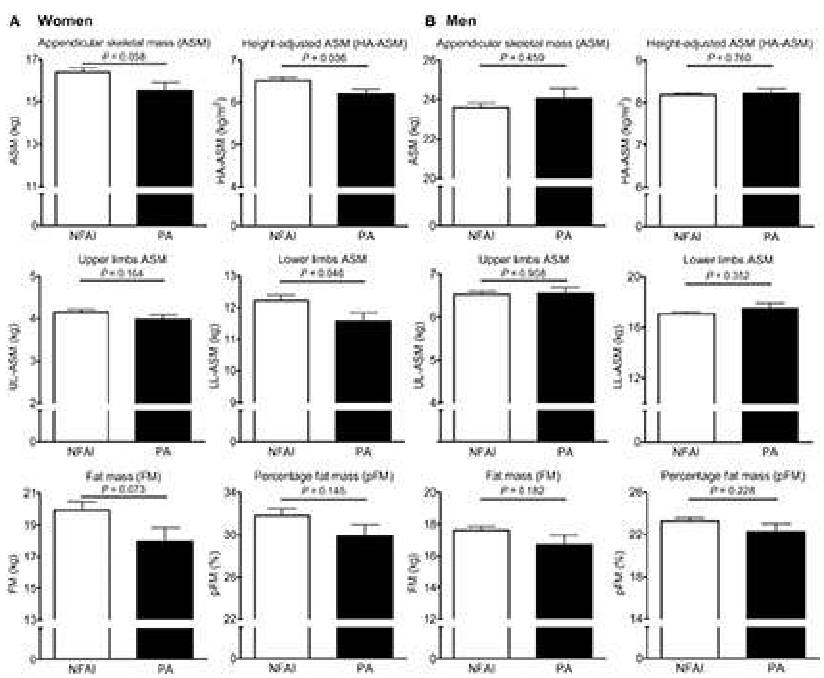

FIGURE 2. Differences in appendicular skeletal muscle mass (ASM), height-adjusted ASM (HA-ASM), upper limb ASM (UL-ASM), lower limb ASM (LL-ASM), fat mass (FM), and percent FM (pFM) between subjects with and without primary aldosteronism (PA). **(A)** is for women and **(B)** is for men. Values represent estimated means, with 95% confidence intervals calculated from analysis of covariance (ANCOVA) after adjusting for age, menopausal status in women, body mass index, regular outdoor exercise, alcohol intake, current smoking, mean arterial pressure, glomerular filtration rate (GFR), and K^+ levels. NFAI, non-functioning adrenal incidentaloma.

Finally, we performed multiple logistic regression analyses to identify any association between PAC or the presence of PA and the risk of lower skeletal muscle mass (Table 3). For women, the odds ratio (OR) [95% confidence interval (95% CIs)] per quartile increase in PAC for lower skeletal muscle mass was 1.18 (1.01–1.39). In addition, the OR of the association between PA and lower skeletal muscle mass in women was 10.63-fold higher (95% CI, 0.83–135.50) than that for the association between NFAI and lower skeletal muscle mass (Table 3). A ROC curve analysis performed to determine the PAC threshold for predicting low skeletal muscle mass in women revealed an AUC of 0.734 (95% CI, 0.639–0.875) (Supplementary Figure 3). The cutoff value, which corresponded to Youden's index (20), was 29 ng/dL. A PAC value ≥29.0 ng/dL predicted low skeletal muscle mass with a sensitivity of 57.1% and a specificity of 92.9%. For women, the OR (95% CI) of the association between PAC values ≥29.0 ng/dL and low skeletal muscle mass was 139.17 (2.40–8069.74).

Table 3. Multiple logistic regression analyses to determine the odds ratio (OR) and 95% confidence intervals (95% CIs) for the association between lower skeletal muscle mass* and plasma aldosterone concentration (PAC) or primary aldosteronism (PA).

	Women		Men	
	OR (95% CI)	*P*	OR (95% CI)	*P*
PAC	**1.18 (1.01–1.39)**	**0.035**	1.05 (0.94–1.17)	0.427
PA	10.63 (0.83–135.50)	0.069	2.96 (0.27–32.68)	0.376

Multivariate analysis was adjusted for age, menopausal status in women, body mass index, current smoking, alcohol intake, regular outdoor exercise, mean arterial pressure, glomerular filtration rate, and K^+ levels. Significant results ($P < 0.05$) are shown in bold. Height-adjusted appendicular skeletal muscle mass [HA-ASM (kg/m^2)] was defined as ASM divided by body height in meters squared (ASM/height^2).

**Lower skeletal muscle mass was defined according to height-adjusted ASM (HA-ASM) using a cutoff of <6.75 kg/m^2 for men and <5.07 kg/m^2 for women (1).*

DISCUSSION

The data presented herein reveal an inverse association between PAC and ASM, UL-ASM, LL-ASM, and HA-ASM (after adjusting for potential confounders) in women. This was not the case for men. Consistent with this, LL-ASM and HA-ASM in women (but not in men) with PA were lower than in women with NFAI. Furthermore, women with PA had lower HA-ASM than 1:1 age- and sex-matched controls with NFAI, and tended to have lower HA-ASM than 1:3 age-, sex-, and menopausal status-matched controls without AI. The odds of low skeletal muscle mass were higher according to the PAC and high PAC level in women, but not in men. To the best of our knowledge, this study presents the first clinical evidence that excess aldosterone might contribute to a reduction in skeletal muscle mass, particularly in women.

Despite the lack of a statistically significant association between PRA and parameters indicative of skeletal muscle mass, the finding of an inverse association between PAC and ASM, UL-ASM, LL-ASM, or HA-ASM suggests that excess aldosterone *per se* has a detrimental effect on skeletal muscle mass. Although aldosterone increases Na^+/K^+ pumps activity in skeletal muscle of patients with Conn's syndrome (21), our data reported that aldosterone has deleterious effects on skeletal muscle mass in humans without CHF. Furthermore, this finding is in agreement with those reported in an animal study showing that injecting rats with aldosterone induces apoptosis of myocytes in skeletal muscle (12). Women in the highest PAC quartile (PAC ≥23.6 ng/dL) had a lower HA-ASM than those in the other three quartiles. Also, PAC ≥29.0 ng/dL was associated with low skeletal muscle mass in women. These findings agree with those reported in another study showing that PAC in CHF patients with cachexia was 2-fold higher than that in non-cachectic CHF patients, and more than 3-fold higher than that in age-matched individuals (35.5 ng/dL vs. 18.0 ng/dL and 10.8 ng/dL, respectively), despite the possibility that impaired cardiac function was a confounding factor (11). Taken together, the results of both the previous and present studies suggest the detrimental effects of aldosterone excess on the skeletal muscle mass in subjects with a high PAC. Indeed, several studies suggest that spironolactone prevents the loss of skeletal myocytes in animals (12), improves vascular endothelial function and muscle blood flow in patients with CHF (13), and improves muscle contractile performance by increasing magnesium levels and by up-regulating Na^+/K^+ pumps in skeletal muscle of patients with alcoholic LC (8). However, these patients may have experienced muscle wasting due to cachexia from impaired cardiac function or the toxic effects of alcohol. Therefore, our study excluded the combined effects of underlying disease, reported in previous studies and identified the effects of aldosterone excess *per se* on the development of sarcopenia in the general population.

A previous study showed that subjects with NFAI may have a higher risk of atherosclerosis than age-, or sex-matched subjects without adrenal gland lesions, and suggested that the body composition of patients with NFAI may differ from that of subjects without AI (22). Therefore, we compared the muscle mass of PA patients with 1:3 age-, sex-, and menopausal status-matched controls without AI. And we found that HA-ASM in women, but not in men, was lower in patients with PA than in age-, sex-, and menopausal status-matched controls without AI, although with a marginal significance. Therefore, these results also suggest a detrimental effect of PA on skeletal muscle metabolism in humans.

Another interesting finding reported herein is that the deleterious effects of excess aldosterone on skeletal muscle mass occurred only in women, and that it was more evident in the lower limbs than in the upper limbs. The reason for this sex dimorphism is unknown; differences in 11β-hydroxysteroid dehydrogenase (11β-HSD) expression and in PAC according to sex might be subsidiary reasons. First, we speculate about the sexual dimorphism of 11β-HSD. In the skeletal muscle, there are two isoforms of 11β-HSD; 11β-HSD1 (converting inactive cortisone to active cortisol), and 11β-HSD2 (converting cortisol to cortisone), resulting in the protection of MR from cortisol and the regulation of the binding of aldosterone to MR (23, 24). Upregulation of skeletal muscle 11β-HSD1 occurring with age in women, but not in men (24) might act as a local tissue amplifier of cortisol, mimicking aldosterone as an MR agonist, due to the high affinity of cortisol equivalent to aldosterone for MR (25, 26). Second, the 12/106 women (11.3%) and 6/204 men (2.9%), P = 0.003, with high PAC (≥29.0 ng/dL), may be more vulnerable to the deleterious effects of excess aldosterone. ASM in women with PA was 5.4% lower in the lower limbs (P = 0.046), but only 4.3% lower in the upper limbs (P = 0.164), than that in women without PA. This pattern of greater muscle weakness in the lower limbs than the upper limbs in those with PA is similar to that observed in patients with overt hypercortisolism; thus, the issue remains unresolved (27).

Although it is assumed that old age starts at about 65 years of age, several studies showed that age-dependent loss of skeletal muscle mass starts in middle-aged adults between 45 and 65 years of age (28, 29). In line with the results of a previous study, decreased HA-ASM in Korean men accelerated after 40 years of age, and that in Korean women began after around 55 years in a study on the assessment of muscle mass in Koreans, using the Korea National Health and Nutrition Examination Survey IV (30).Therefore, our study showed the effects of aldosterone excess on skeletal muscle mass in the early phase of aging-related loss of skeletal muscle mass. Generally, the secretory functions of hormones fall with aging. In line with the results of previous studies showing that PAC decreases with age (31, 32), we also showed the inverse association of PAC with age. However, these results could not exclude the inappropriate activation of

MR, as well as the efficacy of pharmacological MR antagonism therapy in aging populations (31). Since there is no report about the role of aldosterone/MR on skeletal muscle function in aging-related skeletal muscle mass loss in humans, further studies in those aged over 65 years and long-term follow-up period will be needed.

A major strength of this study is that we minimized selection bias by screening consecutive subjects with newly diagnosed AI. Also, we analyzed patients with PA as an ideal human model to explain the effect of excess aldosterone on skeletal muscle. However, the study has several limitations. First, the accuracy of BIA readings is affected by variable parameters such as body temperature, position, and hydration status (1, 33). However, because BIA is reproducible, inexpensive, and easy to use, and it has been validated for sarcopenia diagnosis (1, 33), the Asian Working Group for Sarcopenia regards the method as suitable for measuring muscle mass (1). Second, we did not measure physical performance or muscle strength. When diagnosing sarcopenia, it is essential to identify reductions in muscle function (physical function or muscular strength), not only muscle mass (1, 33). Neither physical performance nor muscle strength was measured in our cohort; we analyzed only the lower skeletal muscle mass in patients with PA. Therefore, other parameters including physical performance or muscle strength should be measured to enforce our findings of the hazard effect of muscle loss in patients with PA in future studies.

In summary, women with PA had lower skeletal muscle mass than those with NFAI, suggesting that excess aldosterone has an adverse effect on skeletal muscle. Further studies are required to identify the complex mechanisms underlying the marked increase in aldosterone concentration and sarcopenia in aging humans.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the Supplementary Files.

AUTHOR CONTRIBUTIONS

JHK and SHL contributed equally to this study. JHK and SHL had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. MKK, S-EL, YYC, and SS conception or design of the work. MKK and S-EL analysis or interpretation of data for the work. B-JK, K-HS, and J-MK acquisition of data for the work. JHK, SHL, B-JK, K-HS, and J-MK drafting of the work or revising it critically for important intellectual content. MKK, S-EL, YYC, SS, B-JK, K-HS, JHK, J-MK, and SHL final approval of the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00195/full#supplementary-material

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REVIEW
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Neuroimaging and Neurolaw: Drawing the Future of Aging

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Human brain-aging is a complex, multidimensional phenomenon. Knowledge of the numerous aspects that revolve around it is therefore essential if not only the medical issues, but also the social, psychological, and legal issues related to this phenomenon are to be managed correctly. In the coming decades, it will be necessary to find solutions to the management of the progressive aging of the population so as to increase the number of individuals that achieve successful aging. The aim of this article is to provide a current overview of the physiopathology of brain aging and of the role and perspectives of neuroimaging in this context. The progressive development of neuroimaging has opened new perspectives in clinical and basic research and it has modified the concept of brain aging. Neuroimaging will play an increasingly important role in the definition of the individual's brain aging in every phase of the physiological and pathological process. However, when the process involved in age-related brain cognitive diseases is being investigated, factors that might affect this process on a clinical and behavioral level (genetic susceptibility, risks factors, endocrine changes) cannot be ignored but must, on the contrary, be integrated into a neuroimaging evaluation to ensure a correct and global management, and they are therefore discussed in this article. Neuroimaging appears important to the correct management of age-related brain cognitive diseases not only within a medical perspective, but also legal, according to a wider approach based on development of relationship between neuroscience and law. The term neurolaw, the neologism born from the relationship between these two disciplines, is an emerging field of study, that deals with various issues in the impact of neurosciences on individual rights. Neuroimaging, enhancing the detection of physiological and pathological brain aging, could give an important contribution to the field of neurolaw in elderly where the full control of cognitive and volitional functions is necessary to maintain a whole series of rights linked to legal capacity. For this reason, in order to provide the clinician and researcher with a broad view of the brain-aging process, the role of neurolaw will be introduced into the brain-aging context.

Keywords: neuroimaging, neurolaw, aging-brain, geriatric endocrinology, vascular risk factors, Alzheimer's disease, magnetic resonance imaging, positron emission tomography

INTRODUCTION

The diagnosis and management of age-related brain cognitive diseases (ABCDs) leading to dementia are undergoing major changes in terms of concepts and technological progress (1). In recent years, it has become evident that it might not be necessary to accept the stereotype of aging as an unalterable process of decline and loss. As life expectancy increases further in the coming decades, the goal for the coming years should be an extension of healthy life combined with a full range of functional and mental capacities in the very late stages of life. With this goal in mind, the development of neuroimaging in recent decades has opened new perspectives in clinical and basic research on brain aging. Structural, metabolic, functional and molecular neuroimaging currently plays a pivotal role in the definition of the individual's brain aging in every phase of the physiological and pathological process (i.e., normal, preclinical, prodromal and dementia state for Alzheimer's disease, AD). Structural neuroimaging (such as computed tomography, CT, and magnetic resonance imaging, MRI) is used in clinical daily activity to detect aging-brain co-morbidity factors, such as vascular disorders, related to modifiable lifestyle risk factors and to help us to adopt preventive therapies. Abnormalities in structural MRI, such as hippocampal volume decrease, are clearly detectable before clinical signs and thus represent one of the most reliable structural imaging markers for AD (2). A multimodal MRI approach, combining different MRI techniques, has been successfully used to identify normal brain aging (3) and preclinical/early signs of neurodegenerative aging (4, 5). In the research field of ABCDs, functional neuroimaging (such as functional magnetic resonance, fMRI) has provided evidence of considerable brain plasticity. The functional connectivity approach provides an invaluable resource for comparing and understanding the changes that occur between healthy brain aging and neuropathological conditions, such as dementia (6). Finally, metabolic and molecular biomarkers of brain functional impairment, neuronal loss and protein deposition, which can be assessed by means of positron emission tomography (PET), are increasingly being used to diagnose AD in research studies and in qualified memory clinics (7).

It is thus evident that neuroimaging enhances knowledge of the many aspects that revolve around ABCDs and should encourage us to think “out-of-the-box” and to develop broader perspectives of this phenomenon. In a wider perspective, the neuroimaging information available needs to be combined with the identification of common risk factors in the elderly so as to prevent and to delay age-related brain cognitive physiological and pathological changes. Frailty is the term that most accurately describes this condition that affects the elderly and is characterized by loss of biological reserves, failure of homoeostatic mechanisms and vulnerability to adverse outcomes. Although endocrine changes related to brain aging and to ABCDs are not normally included in the set of influencing factors, they are considered particularly important in frailty because of complex inter-relationships with the brain, immune system and skeletal muscle. Moreover, endocrine diseases, such as thyroid dysfunction, are common clinical issues that affect an aging population. The optimal diagnosis and management of these diseases are paramount to improve the health care and quality of life of patients and to reduce the economic burden of an aging population.

Neuroimaging may be essential for the correct management of ABCDs not only within a medical but also a legal perspective, according to a broad approach based on the development of a relationship between neuroscience and other disciplines that has given rise to a series of neologisms i.e., neuroanthropology, neurophilosophy, neuropolitics, neuroeconomics, neurosociology, neuropsychology, neuroethics and neurolaw (8). Neurolaw is an emerging discipline that deals with various issues related to the impact of neurosciences on individual rights. In this regard, enhancing the detection of physiological and pathological brain aging by means of neuroimaging may make a major contribution to the field of neurolaw. Indeed, as the elderly individuals usually carry out daily activities that require the full control of cognitive and volitional functions, they need to be aware of their abilities and limits so as to avoid affecting their own legal interests as well as those of the people they are surrounded by. This applies even more so to the field of public security, since carrying out activities that pose a risk to oneself and others requires the ability to observe the precautionary rules required to guarantee an adequate balance between social costs and benefits.

Given these premises, the aim of this article is to provide a current overview of the physiopathology of brain aging and of the role and perspectives of conventional and advanced neuroimaging techniques in this context. When the process involved in ABCDs is being investigated, factors that might affect this process on a clinical and behavioral level (genetic susceptibility, risks factors, endocrine changes) cannot be ignored but must, on the contrary, be integrated into a neuroimaging evaluation to ensure a correct and global management, and they are therefore discussed in this article. Finally, in order to provide the clinician and researcher with a broad and multidimensional view of the brain-aging process, the role of a more recent discipline, i.e., neurolaw, will be introduced into the ABCDs context.

What Is Aging-Brain Cognitive Disease?

The age-related brain process, including the progressive loss of cognitive functions, has traditionally been considered to be physiological and unavoidable. The maturation and physiological aging of the nervous system is an inescapable process that is required for the progressive adaptation of the individual and may be considered the basis of a positive vision of aging. Starting from fetal development, throughout life there is a constant adaptation of the nervous system to internal biological modifications and the external environment designed to improve and maintain adequate levels of performance. These changes are characterized by processes of proliferation and neuronal migration, of axonal and dendritic branching and myelination, and of formation and elimination of synapses. In childhood, the cortical regions are mainly developed for motor and sensory functions; in adolescence, the frontal and prefrontal cortices are implicated in higher cognitive functions, while the subcortical structures (amygdala, striatum) modulate the stimuli by means of social, adversative, and emotional values (9).In adulthood, the brain continues to undergo progressive structural microscopic (widespread reduction of neurons and oligodendrocytes, reduction of myelinated fibers), macroscopic (reduction of cerebral volume and cortical thickness, enlargement of the liquor spaces, of sulci and of the ventricular system) and functional changes (in the connectivity of neural networks). These biological changes in the adult brain underlie the processes of successful physiological aging. Successful aging does not merely mean lengthening the life span, but doing so with a low risk of illness and disability as well as with the preservation of mental and psychosocial capacities (physical activity, leisure, fun, interpersonal relationships). Pathological aging, on the other hand, is the condition in which one's biological and chronological ages do not coincide, to the disadvantage of the former. The pathophysiology of many neurodegenerative syndromes, of which AD is the foremost, is complex and lacks any isomorphism between the clinical manifestations and underlying pathogeny. It includes a number of different mechanisms related to genetic, molecular (misfolding proteinopathies), vascular and inflammatory processes. From a biological point of view, in AD the accumulation of abnormal proteins in the brain (neurons and/or glia) consisting of extracellular deposits of ß amyloid (Aß), which is insoluble and toxic in the cerebral cortex and cortical and leptomeningeal artery walls, and of neurofibrillary aggregates (tau) (intraneuronal deposits of tau protein) induces a diffuse cascade of intracellular metabolic disturbances, abnormal microcirculation, and pathogenic recruitment of the central nervous immune system. Selective hippocampal neuronal vulnerability is the basis of AD in the initial phase, in which degeneration propagates to brain regions that will be spared by the pathological process until the later stages of disease (for example the cerebellum) (10).The kinetics of neurodegeneration is a slow process with a clinical silent phase that may last decades. This clinically silent phase is defined as the preclinical phase of the disease. The nervous system's response to progressive tissue damage translates into complex endogenous plastic mechanisms that tend to preserve cognitive functions over time, before the clinical onset of a disease, such as behavioral compensatory phenomena and neuronal plasticity accompanied by the activation and remodeling of parallel circuits, remapping of cortical areas, neurogenesis and angiogenesis (11). Mild cognitive impairment (MCI), the first clinical phase of ABCDs, is a syndrome of acquired cognitive impairment not associated with any functional limitations that has heterogeneous presentations and underlying pathologies; up to two thirds of subjects with amnestic MCI have underlying AD pathology while the remainder exhibit normal age-related changes. The prodromal stage of AD is the phase in which symptoms have become manifest but before disability is apparent (7).

In this regard, the Anglo-Saxon expression “time is brain,” which typically refers to the acute treatment of cerebral ischemia aimed at saving neurons affected by the pathological event as rapidly as possible, assumes importance even in AD. Indeed, just as an intervention within hours of the onset of a stroke (the therapeutic window) makes it possible to salvage damaged tissue, an earlier intervention in neurodegenerative diseases such as AD may slow down the progressive neuronal loss and make the therapeutic treatment potentially effective. The use of neuroimaging biomarkers that identify elderly subjects in the preclinical or early phase of AD when disease-modifying therapies might be most effective is of considerable interest (12).

Which Factors Influence the Evolution of Brain Aging?

Human brain-aging is a complex, multidimensional phenomenon (Figure 1). Neuroimaging alone cannot provide a complete description of the age-related brain processes but must be supplemented with knowledge of the numerous factors and phenomena that might affect these processes in order to help the clinician to maintain biological reserves and homoeostatic mechanisms in the elderly and prevent and treat early pathological phenomena of brain aging.

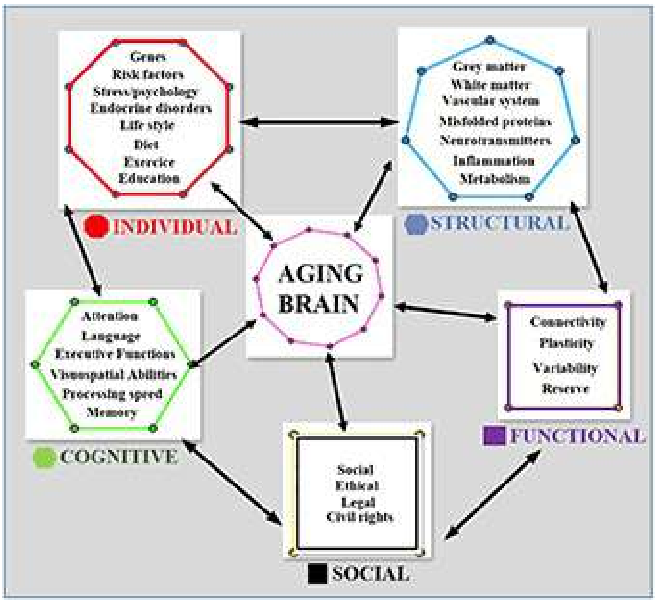

FIGURE 1. A multidimensional geometric model of cognitive brain aging. Each geometric figure contains the set of factors that affect the multidimensional phenomenon of aging. The number of sides of each geometric figure corresponds to the number of factors contained in it, e.g., the hexagon contains the 6 main cognition factors. Bidirectional arrows indicate an effect of the factors upon each other and on the aging phenomenon.

A greater degree of structural and functional aging may be due to genetic predisposition (e.g., hetero-homozygosity for Apolipoprotein E ε4—APOE4), to some diseases (e.g., small vessel disease, amyloid angiopathy, endocrine disorders, acquired brain injury), to medical treatment (e.g., chemotherapy, radiotherapy) or to advanced age.

There is a significant heterogeneity among the elderly in the rate of decline in some cognitive functions, such as perceptual reasoning and processing speed. Individual differences may also be lifestyle-related and be linked to higher levels of physical fitness, cognitive stimulation and societal investments in a safe and healthy environment, as well as to other factors that help to preserve cognitive function. Morbidity may be prevented and controlled in part by healthy lifestyle measures, which appear to decrease the prevalence of long-term disability in the elderly. Nevertheless, the varying effects of aging on the brain structure, metabolism and function have multiple complex etiologies that are often difficult to identify early in life.

Genetically, ApoE is a major cholesterol carrier that supports lipid transport and injury repair in the brain. APOE polymorphic alleles are the main genetic determinants of AD risk: individuals carrying the ε4 allele are at a higher risk of AD than those carrying the more common ε3 allele, whereas the ε2 allele reduces the risk. The presence of the APOE ε4 allele is also associated with an increased risk of cerebral amyloid angiopathy and ABCDs (13). It has recently been shown that the association of the APOE ε4 allele, high Aβ levels (measured by PET) and increasing age affects memory decline in non-demented elderly subjects and can be used to estimate the risk of memory decline (14).

Among the primary pathological factors that can affect brain aging, vascular phenomena are known to play a prominent role. A growing body of evidence points to an early modulatory role of vascular factors in the genesis and development of pathological brain aging, e.g., in late-onset AD (15). A cerebrovascular dysregulation had been consistently found as a primary pathological factor in the genesis and progression of late-onset AD (16, 17). Vascular activity plays an active role on misfolded protein deposition and clearance mechanisms and complex multifactorial interactions conducive to AD. Moreover, there is a large body of evidence that points to a direct link between vascular risk factors and AD (18). Hypertension, diabetes, hyperhomocysteinemia and dyslipidemia are some of the pathological factors that increase the possibility of stroke and ischemia or at least lead to development of cerebral small vessel disease (19).

In addition to the prominent role of cerebral vascular dysfunction and risk factors in the onset of ABCDs, it is important to bear in mind the pathological role played by misfolded proteins, particularly by Aß. The effects of Aß and T toxicity have been causally linked to brain oxidative stress (20), mitochondrial dysfunction (21), synapse and spine loss (22), widespread neuronal dysfunction and death (23), and synaptic plasticity impairment (24).

The significant heterogeneity among the elderly in the speed at cognitive decline progresses suggests that a combination of several factors is required to induce the gradual brain damage that leads to the clinical onset of AD.

The endocrine changes related to brain aging and to the pathophysiology of ABCDs are not normally included in the set of influencing factors. Since the brain may be considered as an endocrine gland, endocrine system disorders in the brain affect its development and evolution. The literature in this field highlights a potential role of endocrine changes in the progression of ABCDs. For example, several somatic and lifestyle factors associated with AD, including hypertension, obesity, diabetes, physical inactivity and smoking, are reported to be related to endocrine changes (25). These factors are unlikely to occur on their own but might interact in a synergistic or antagonistic way or form clusters (e.g., metabolic syndrome). During aging, the secretory patterns of hormones produced by the hypothalamic-pituitary axis change, as does the sensitivity of the axis to negative feedback by end hormones. Moreover, glucose homoeostasis tends toward disequilibrium as age increases. For both males and females, an age-related loss of sex steroid hormones has been associated with an increased risk of cognitive decline (26). Aging-induced effects are difficult to disentangle from the effects of other factors that are common in the elderly, such as chronic diseases, inflammation and low nutritional status, all of which can also affect the endocrine systems. Finally, neurogenesis can be affected by several factors, including the release of growth factors, estrogen, and glucocorticoids. Taken together, these observations suggest that the endocrine system may be involved in the evolution of ABCDs.

Since this vast and complex field of research does not fall within the scope of this article, it shall not be dealt with extensively here. However, the close relationship between the endocrine system and brain aging deserves to be mentioned.

Sex hormones are known to be a fundamental factor that influences the brain from the earliest stages of life. Sex hormones (estrogens, androgens, and luteinizing hormone) and gonadotropins not only impact the non-reproductive domains of the brain and human behavior but are influential in maintaining neuronal health and promoting neuronal cascades that underpin cognitive processes. Indeed, steroid hormone and gonadotropin receptors are present in many brain areas, including the hippocampus and frontal cortex, both of which play a critical role in memory functioning (27). Ovarian hormones are known to influence several factors in the brain, including growth factors (e.g., neurotrophins), the inflammatory and immune response, mitochondrial function and the cholinergic system. This system requires the neurotransmitter acetylcholine, which is a key regulator of learning and memory consolidation. Cholinergic neurons projections from the basal forebrain synapse onto ?-aminobutyric acid (GABA)ergic cortical neurons; GABA is the primary inhibitory neurotransmitter in the brain and an important neuromodulator for cognitive processes, including hippocampal and cortical function. Inhibitory GABAergic neurons and signaling become dysregulated with aging (28).

Both natural menopause, which is characterized by fluctuating and decreasing levels of estrogens and progesterone and an increase in serum gonadotropin follicle-stimulating hormone (FSH), and surgical menopause, which is induced by removal of the ovaries, have been associated with cognitive complaints, particularly in the area of memory (29), with an increased risk of cognitive impairment and dementia later in life (30). Moreover, both types of menopause may lead to medial temporal lobe structural abnormalities later in life (31). By contrast, the risk of AD does not increase among women who commence hormone therapy following premenopausal oophorectomy and continue this therapy until the natural age of menopause (32). Lastly, sex hormones also modulate the impact of genetic risk factors in the etiology of AD, such as the APOE ε4, the strongest known genetic risk factor for late-onset AD, thereby resulting in a higher risk for AD conversion in females than in males (33).

In addition to changes in sex hormone production, the most significant age-associated endocrine change resides in the hypothalamic-pituitary-adrenal (HPA) corticotropic axis (34), a major component of the stress response system, which may lead to or accelerate hippocampal impairment (35). Stress has repeatedly been shown to exacerbate symptoms and accelerate disease onset in AD (36, 37). Acute stress activates HPA and the sympathetic nervous system, which in turn increases the release of glucocorticoids and catecholamines (38). These molecules initiate a neuroendocrine response, mobilizing lipids, glucose and other resources in order to facilitate cognitive and physical demands. However, in conditions of acute psychological stress, these neuroendocrine responses are not linked to an increased metabolic demand. In chronic stress, this prolonged activation of the stress system has been linked to a large number of comorbidities ranging from metabolic dysfunction and cardiovascular disorders to cognitive dysfunction and psychological disorders, such as depression (39). Finally, as stress is a risk factor for AD and women are twice as likely to develop mood disorders where stress is a major etiology, sex dimorphism in stress responses may explain the higher incidence of AD among women (40).

Age-related brain changes have been reported to be associated with other endocrine factors, such as insulin (41, 42). High insulin blood levels and insulin resistance has been reported to be important contributors to progressive cognitive impairment and neurodegenerative processes. The maintenance of insulin sensitivity signaling may preserve cognition, which results in the well-being of elderly people (43). Insulin receptors are widely distributed within the brain, with the highest concentrations in the hypothalamus, hippocampus, olfactory bulb, cerebellum, amygdala, and cerebral cortex (44). Central insulin plays a role in maintaining energy homeostasis, as it has the ability to increase blood glucose levels (acting in opposition to peripheral insulin), to decrease feeding and body weight and to lower insulin blood levels (45). Insulin has neuroprotective properties and neurotrophic effects on neurons. It may also positively affect emotion and cognitive processes, including attention, executive functioning, learning, and memory (46).

Normal thyroid function also appears to be an important factor in maintaining optimal cognition in human aging (47). Hypothyroidism, at any age, causes cognition to deteriorate because it prevents the brain from adequately sustaining the energy glucose-consuming processes required for neurotransmission, memory and cognitive functions. Low glucose brain uptake, which is commonly associated with deteriorating cognition and AD, may be present years before clinical evidence of AD appears (48, 49). Since thyroid hormone concentrations change with age and since cognitive decline occurs with aging, physiological changes in thyroid function might be causally related to changes in cognition during normal aging. In view of the potentially increased risk of cognitive decline associated with thyroid dysfunction and considering that progressive cognitive decline is the central clinical feature of AD, it is conceivable that thyroid status contributes, at least in part, to the clinical manifestation of AD. Indeed, several clinical reports and laboratory and epidemiological studies point to a link between thyroid hormones and AD pathophysiology (50, 51).

Knowledge of the complex interactions within this endocrine-aging-brain triad is growing in breadth and depth as scientific discoveries are made. It will be possible to gain new insights by continuing these investigations into how these paths meet and affect each other. Clarifying the changes associated with aging in these molecular mechanisms and the hormonal milieu in a systematic and demonstrable fashion is likely to shed light on how and when the brain responds to endogenous hormone changes and to potential exogenous hormone treatment.

How Can Neuroimaging Be Used to Detect Brain Aging?

Neuroimaging is the set of diagnostic and experimental methods used for visualizing to the structural, functional, metabolic, and molecular features of the human brain *in vivo*. Neuroimaging technology is at the forefront of advances in both our understanding of the brain and our ability to diagnose and treat brain diseases. Since CT, we have moved on to multimodal MRI studies, to the development of molecular imaging techniques (PET) and, more recently, to hybrid scanners (PET/CT, PET/MRI). Hybrid scanners, which are still relatively scarce, represent the latest imaging technology that offers a multidimensional evaluation of the central nervous tissue. In PET/MRI hybrid imaging, each voxel (i.e., the unit that makes up the three-dimensional or volumetric image) contains structural, metabolic, molecular and functional data that provide more accurate and complete information on the nervous tissue *in vivo* (51). Hybrid imaging is the latest tool available to address the aging brain and human neurodegenerative diseases (Figure 2).

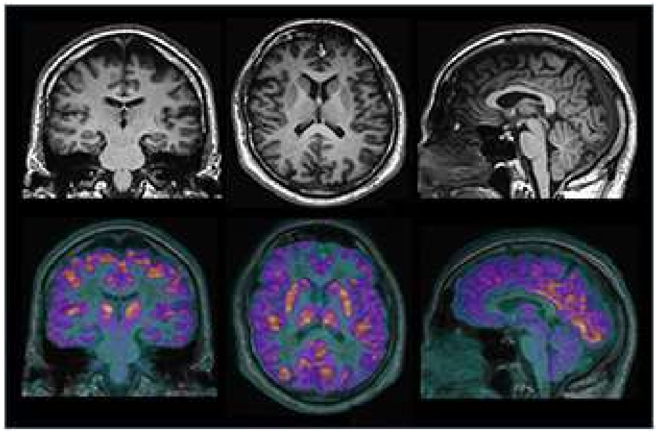

FIGURE 2. Hybrid brain PET/MRI imaging (Biograph mMR, Siemens) in a normal subject. The top picture shows anatomical MRI 3D T1-weighted images on the coronal, axial and sagittal slices. In the figure above, a metabolic 18FDG PET image of the same individual is combined with the MRI image to obtain information on the morphology, anatomy and metabolism of the brain.

In daily clinical practice, when ABCDs is suspected, a neuroimaging examination is aimed at either supporting or ruling out the diagnosis of dementia, of alternative etiologies (small vessel disease, metabolic, and endocrine pathologies, etc.) or signs of vascular co-morbidity (amyloid angiopathy). Within this context, CT remains the most accessible and widespread technique and is often the first examination to be requested by the clinician. However, MRI has, owing to its greater sensitivity in the study of the brain, become the elective technique, especially in the early stages of AD. Conventional MRI can be used to structurally and temporally characterize the vascular lesions and quantify the lesion load present in the aging brain (Figure 3). In addition to excluding other brain diseases, such as tumors and infectious and inflammatory diseases, MRI provides two pieces of critical information: it shows whether there is any chronic vascular damage, which is included in the differential diagnosis of dementia, and it qualitatively assesses brain atrophy by highlighting any enlargement in the perivascular and subarachnoid spaces. On MRI, neuronal loss corresponds to a volume reduction (atrophy) that can be seen and quantified at an early stage of the disease at the level of the mesial temporal region, and in particular in the entorhinal and hippocampal regions (52). A visual assessment has proven to be specific in the differential diagnosis between AD and other dementias, particularly if combined with a neuropsychological assessment (53). However, when assessing individuals, a visual estimation of brain atrophy in the early stages may not be sufficiently sensitive or specific because atrophy occurs when signs of dementia are already present. The MRI volumetric technique is a relatively simple method that allows accurate estimates of regional volumes and has been extensively used in brain-aging studies [for review see (54)]. However, in the early phase of AD, the sensitivity and specificity of MRI hippocampal volumetric measurements may not exceed the maximum accuracy value of 80%, whereas it is considerably more useful in the longitudinal evaluation of the degenerative process (52). Recently, early MRI structural abnormalities in the neocortex and cortical thickness have aroused growing interest (55, 56). Reduced gray-matter volume in the posterior cingulate and/or precuneus and hippocampus (57), prefrontal cortex (58) and parietal lobe (59) has been described in cognitively intact individuals before progression toward mild cognitive impairment (MCI).

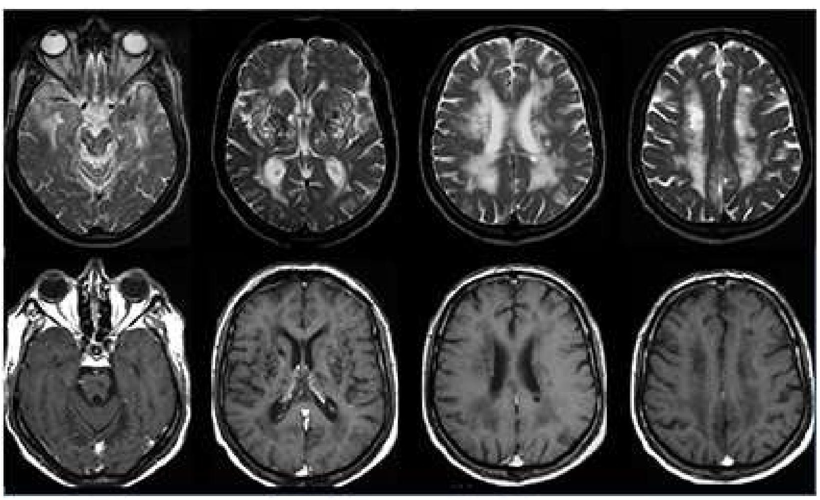

FIGURE 3. Neuroimaging of cerebrovascular disease in the aging brain. The figure shows a brain MRI study of a patient with risk factors affected by cognitive disorders. The images above (T2-weighted axial slices) and below (T1-weighted axial slices, after contrast enhancement) show diffuse, punctate deep white matter foci, hyperintense T2-weighted images, with a low signal intensity on T1-weighted images and without contrast enhancement, suggesting cerebral small vessel vascular disease.

Differences in the extension and signal intensity of cortical activation in task-based fMRI have been observed between MCI patients, AD patients and control groups (60). fMRI might provide important information for the assessment of disease progression in groups and predict neuromodulation as well as the effects of drugs. It may not, however, be easy to transfer group analysis results into daily clinical practice for individual subjects.

Resting-state functional connectivity between the hippocampus and the posterior part of the default mode network is significantly reduced in AD patients (61). There is, however, as yet insufficient evidence of a distinct pattern of changes in functional connectivity that may be used as a predictor of further progression to clinical AD.

PET with 18FDG (fluoride radioisotope 18 combined with the deoxyglucose molecule) can reveal, from the early phase of AD, a focal reduction in glucose metabolism and be used to make a differential diagnosis with cognitive brain aging or other forms of dementia (Figure 4). PET combined with the use of specific tracers that bind to Aß deposits, such as the isotope 11CPiB (called the Pittsburg compound B), and subsequently by using various 18F-amyloid-binding ligands with a longer half-life, has been proposed as a technique for the preclinical and early diagnosis of AD. The accumulation of Aß amyloid, measured in PET, correlates with the histological findings of Aß distribution in normal aging and AD. The accumulation of Aß begins many years before the onset of symptoms and represents a preclinical phase of AD in asymptomatic subjects and a prodromal phase in those with MCI. More recently, a number of PET tracers that target *in vivo* tau fibrils have been developed (62). The PET tracer [18F] flortaucipir allows the *in vivo* quantification of paired helical filament tau, a core neuropathological feature of AD. Tau deposition, as measured by the 18F PET tracer, significantly correlates with cortical thickness. Recent reports have shown a relationship between increased tau tracer uptake and worsening cognitive status (63, 64). Using neuroimaging criteria based on Aß and tau PET data, AD has recently been defined by the positivity of biomarkers of both amyloidopathy (A1) and tauopathy (T1), which is in keeping with the pathological definition of the disease.

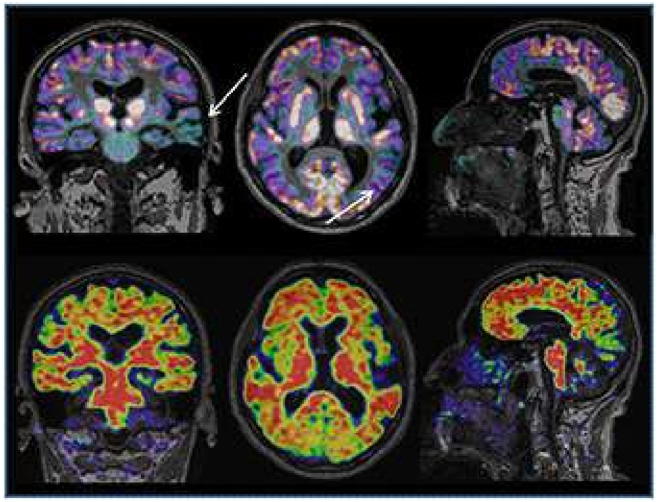

FIGURE 4. Hybrid PET/MRI imaging (Biograph mMR, Siemens). The picture above shows MRI 3D T1-weighted and 18FDG PET coronal, axial and sagittal slices in a patient affected by progressive speech disorder. The combined structural and metabolic image shows focal atrophy and reduced glucose metabolism in the left temporal lobe (white arrows), suggesting a diagnosis of Primary Progressive Aphasia, a rare form of dementia. The picture below shows MRI 3D T1-weighted and a 18F-flumetamole PET coronal, axial and sagittal slices in the same patient. The combined structural and molecular (Aß amyloid accumulation) image shows a diffuse increase in Aß amyloid deposits in the cortex, supporting the diagnosis of brain neurodegenerative disease.

These PET biomarkers have led to the proposal of the term “preclinical AD” when the risk is particularly high (e.g., both Aß and tau markers exceed the pathological thresholds) and “asymptomatic at risk for clinical AD” (AR-AD) when the evolution to clinical AD is less likely or has yet to be confirmed (only one pathophysiological PET marker considered abnormal) (65). A combination of the clinical criterion, which is related to the cognitive domain of memory, and a multimodal approach based on cerebrospinal fluid (CSF concentrations of tau and Aß42) and neuroimaging biomarkers (PET - 18FDG, PET Aβ and tau, MRI volume of the hippocampus and cortical thickness) will play a decisive role in large-scale drug trials of preclinical and prodromal AD. However, since the predictive performance of the multimodal approach has yet to be fully established, findings should be assessed according to their sensitivity and/or specificity and their condition (i.e., isolated or in combination) (7, 66).

Although AD is the most common cause of major cognitive disorders, accounting for 60% or more of all dementias, a clinical diagnosis of probable AD has a sensitivity and specificity of only 70.9 and 70.8%, respectively, when compared with the “gold standard” pathological findings (67). It is for this reason that a considerable effort has been made in recent years to assess the analytical and clinical validity of biomarkers related to neurodegeneration, such as neuroimaging and CSF, so as to be able to translate them from research into clinical practice (68). The use of neuroimaging biomarkers may be challenging for clinicians, particularly in patients with ABCDs. Moreover, the fact that the clinical usefulness of these biomarkers has yet to be fully ascertained is hampering the reimbursement for these tests by health insurance providers, their widespread clinical implementation and, consequently, improvements in the quality of health care. A strategic roadmap to foster the clinical validation of biomarkers in AD has provided sufficient evidence of the analytical validity of all biomarkers (phase 1), whereas their clinical validity (phases 2 and 3), and utility (phases 4 and 5) have yet to be proven. Research priorities aimed at completing these phases include the standardization of the readout of these assays and of normality thresholds, the evaluation of their performance in detecting disease early, the development of diagnostic algorithms comprising combinations of biomarkers, and the development of clinical guidelines for the use of biomarkers in qualified memory clinics (69).

Very recently, the evaluation of the clinical utility of a single biomarker for the diagnosis of ABCDs, has yielded interesting data regarding the accuracy of the diagnosis and prognosis. FDG, the most widely available PET radiotracer, has been shown to support the diagnosis of AD in MCI subjects with an accuracy ranging from 58 to 100%. The pattern of hypometabolism in the posterior cingulate and posterior temporo-parietal areas that characterize the conversion from MCI to AD is considered helpful in the diagnosis of AD in MCI subjects. An MCI constellation is challenging if diagnosed solely on clinical grounds with regard to outcome prediction because declining memory is also a feature of normal aging, and some MCI cases may never progress to the dementia stage or may even reverse to normality. Therefore, one of the main advantages of FDG-PET over other biomarkers (i.e., amyloid imaging or CSF) lies in its high predictive value for short-term conversion to AD in MCI subjects, which in turn offers clinically relevant prognostic information (70). Evidence regarding the clinical routine use of FDG-PET as a means of detecting diagnostically meaningful early signs of neurodegeneration in asymptomatic subjects with an increased risk for AD, as defined by subjective cognitive decline, cerebral amyloid-pathology or APOE4-positive genotype, is still limited (71).

With regard to the clinical role of the biomarker Aβ-PET, a recent search of the literature has shown a significant impact on both the diagnosis and management in MCI subjects and patients with dementias who are referred to memory disorders specialty clinics. The performance of Aβ-PET, used according to criteria (AUC) published to help clinicians to maximize the utility of Aβ-PET (72), yields a higher percent change in the diagnosis than when Aβ-PET is not used, according to the AUC. Beneficial changes increase the diagnostic accuracy and help to ensure patients and families go on to attend a general practice. Changes in management include modified treatment, fewer additional diagnostic tests, different family and patient advice based on the findings and, in some cases, entry into clinical trials (73, 74). Both amyloid-positive and amyloid-negative results are also closely associated with changes in the diagnosis and treatment in both patients with and those without dementia (75). Similarly, a recent study designed to evaluate the impact of Aβ-PET on the diagnosis and management of AD patients in the memory clinic showed that clinical MRI features suggestive of AD predict a positive Aβ-PET scan. Moreover, among patients with MRI features suggestive of AD but with atypical clinical features of AD, the clinical impact on the diagnosis and management was shown to be greater for amyloid negative than amyloid positive Aβ-PET scans (76).

Among patients with established diagnoses at a memory disorder clinic, [18F] flortaucipir PET, which quantifies the paired helical filament tau, has proven highly accurate (sensitivity and specificity) as a means of discriminating AD from other neurodegenerative diseases. Structural MRI measures correlate with PET-tau tracer 18F-AV-1451 in a spatially local manner. This correlation is stronger for longitudinal than for cross-sectional measures of cortical thickness as well as for subjects with cerebral amyloid than for those without, thereby supporting the notion that *in vivo* measures of tau pathology are closely linked to the speed of neurodegenerative change (77). However, the diagnostic performance of the PET-tau tracer is lower in MCI due to AD (78). Lastly, the limited body of evidence on the relationship between tau and cognition in normal aging suggests that the mere presence of tau is not sufficient to cause cognitive changes (79). The PET-tau technique still requires a considerable amount of validation work, including the optimization and standardization of methodological aspects. Although it may be possible to incorporate this technique into clinical trials on a range of subjects with the whole spectrum of AD, the accuracy and potential clinical utility of PET-tau tracer in ABCD subjects require further research in clinically more representative populations (80).

Finally, a recent neuroimaging study aimed to explore the joint relationships of imaging biomarkers (MRI cortical thickness, Aβ-PET, and PET-tau) and cognition, in a cohort of non-demented individuals, using a machine learning model, has shown how the dysfunction of memory process is influenced by the confluence of these three biomarkers: Aβ and tau elevations and lower levels of entorhinal cortical thickness (81). Fully integrating more relevant biomarkers in ABCD subjects and accounting for interplay between brain regions is a major computational challenge this field needs to address.

To sum up, neuroimaging provides a wide range of techniques and methods that evaluate the structural, functional and metabolic bases of ABCDs depending on whether they are used for research, clinical, or pharmaceutical purposes, respectively. In daily clinical practice and in the specialist setting, e.g., in memory clinics, structural MRI may support or rule out the diagnosis of dementia (by identifying atrophy, especially using quantitative techniques), of alternative etiologies (i.e., small vessel disease) or of signs of co-morbidity. PET, when based on FDG and Aβ tracers, increases the diagnostic and prognostic accuracy, which in turn clinically impacts the diagnosis and management of MCI subjects and AD patients. Priorities in the use of PET neuroimaging biomarkers remain the standardization of the readout of these assays and of normality thresholds, the evaluation of their performance in detecting early disease in a larger population, the development of diagnostic algorithms based on combinations of biomarkers, and the development of clinical guidelines for the use of biomarkers in qualified memory clinics.

How Does Brain Aging Affect the Elderly Individual's Mental Capacity in Terms of the Law?

The social and political impact of modern neuroscience has become the foundation of new interdisciplinary platforms which bring together doctors, brain researchers, social scientists and professionals from other fields (82). Within this context, technological advances in neuroimaging point to another radical change in the comprehension of brain aging and its pathologies: in the coming years, neuroimaging markers will become part of routine clinical evaluations and will radically transform not only our clinical approach but also our way of understanding the elderly and their relations with society. Indeed, while the primary objective will continue to be the preclinical and/or early diagnosis of dementia and its treatment, other non-clinical aspects will need to be considered, such as legal issues that must be addressed and managed. The use of neuroimaging to identify signs of brain aging establishes, primarily, a responsibility on the part of clinical practitioners in relation to the management of clinical information, particularly in cases where neurodegenerative disease is involved. On receiving a diagnosis of a probable neurodegenerative pathology, even if in the initial—MCI—or the prodromal phase, individuals will have to be informed that they have a high probability of developing a pathology that will result in a progressive loss of cognitive functions and autonomy. This stigma will change the individual's interpersonal relationships, both public and social, and may lead to their isolation; it could give rise to a fear of losing civil rights and privileges (for example, driving vehicles, participation in public life, voting); it may lead to workplace discrimination in relation to the individual's position and the tasks carried out up until that day (83).

Physiological brain aging and the associated progressive cognitive changes do not particularly affect a person's ability to perform simple daily activities, but they can have an impact on more complex activities requiring a high level of attention and capacity to react. For example, older individuals have been shown to be more at risk of being responsible for road accidents than younger ones (84) due to an evident inability to assess their own driving skills objectively or accurately. Furthermore, there are certain professional categories where a reduction in driving ability would take on even greater significance (such as truck and train drivers, pilots, and air traffic controllers) (85).

In addition, some studies have shown that reduction in cognitive functions in the elderly may, if exacerbated by co-morbid factors (risk factors, cerebral small vessel disease, endocrine changes/diseases), lead to an impairment, albeit to a varying extent, in a number of areas such as those related to attention processes and visual perception, executive functions and memory, and inhibition of an automatic response with respect to a new behavior (86). This may cause a decrease in the ability to perform normal daily activities and a total inability to undertake those activities requiring complex functional capacities such as management of one's finances or of pharmacological therapies and driving ability (87).

It must be underlined that in national legal systems there are no laws providing for a general presumption of incapacity for those individuals who have reached a certain age (88), unlike that established by some legal systems in the field of civil and criminal capacity of minors: see, for example, § 2 of German Civil Code (where the fixed minimum age for active legal capacity is 18 years old) and section 19 of German Criminal Code (where the fixed minimum age for criminal capacity is 14 years old), article 2 of Italian Civil Code (18 y.o.) and article 98 of Italian Criminal Code (14 y.o.), section 1 of U.K. Family Law Reform Act 1969 (18 y.o.), and section 50 of U.K. Children and Young Persons

Act 1933 as amended by U.K. Children and Young Persons Act 1963 (10 y.o.). The lack of a presumption of incapacity is due to the structural difference between minors and the elderly: while for the former the established incapacity is associated with a physiological condition of immaturity due to biological-organic, psychological, and socio-environmental factors (89), for the latter there is a progressive physiological loss of cognitive abilities due to a decrease in brain volume and neuronal connections, which are factors that may occur at different times among elderly individuals depending on their genetic makeup, on their quality of life, and on stimuli external to their central nervous system.

The rationality behind the lack of legal presumption regarding incapacity of the elderly can be considered in three different ways.

Firstly, from the point of view of the rationality of legislative choices, setting an age threshold at which mental capacity is deemed to be impaired would be a solely discretionary and questionable choice on the part of the legislator, potentially with little connection to the modern social context, where brain aging in individuals with no pathological causes tends to diminish increasingly on a chronological basis. This is especially true with the middle and upper social classes (90), where it is easier for older people to enjoy better brain health, as a result of them being more able to benefit from health care for therapeutic or enhancement purposes and to take advantage of cultural motivational stimuli and to enjoy the restorative effect on the brain of new technological tools at their disposal.

Secondly, analyzing unreasonable legal consequences *in malam partem*, such a regulatory intervention would create a potentially unreasonable discrimination, resulting in the loss of a whole series of rights linked to legal capacity, such as voting, finalizing valid contracts, drawing up a will, or taking actions potentially involving risk which are normally permitted by law (for example, driving on the road, carrying out private professional activities, or engaging in hobbies such as hunting or fishing): from this point of view, an abstract presumption of mental incapacity would be at variance with the principle of equality and that of respect for human dignity.

Thirdly, in terms of unreasonable legal consequences *in bonam partem*, the aforementioned environmental factors, together with the physiological (for example, genetic) differences between individuals, bring into focus how the introduction of a presumption of mental incapacity for the elderly would be contrary to the principle of individual responsibility: to consider persons lacking capacity merely because they had exceeded a certain age (over-X), would risk excluding them *a priori* from any responsibility for their own actions, with aberrant consequences particularly in the field of criminal law where requirements for social protection are particularly stringent. In fact in this case the over-X elderly individuals would be exempt from punishment for criminal actions committed regardless of any assessment of their effective capacity to understand the disvalue of their conduct, in contrast to the criminal principle of guilt.

On the other hand, the absence of a legal presumption of mental incapacity should be accompanied by a more vigilant awareness and management on the part of the legislator of the aging-related brain cognitive disease (ABCDs) process of the population and the potential conflict between the rights of the elderly and the opposing interests of citizens interacting with the former, primarily all those related to security and public safety: this aspect deserves to be considered in the field of criminal law, which must deal with preventing and penalizing socially harmful behavior.

Given that in European and non-European legislation there is no presumption of mental incapacity for the elderly who have passed a certain age, the sole means of holding them not responsible for any actions carried out against their own interests or those of third parties is by declaring a condition of mental infirmity based on the detection of pathological factors that aggravate the aforementioned physiological conditions of brain aging, on condition that there exists proof that on account of the disease the elderly individual lacks capacity (91). In these cases, the condition of physiological and pathological brain aging would coincide with insanity and would be subject to general norms regulating its impact on active legal capacity, in civil law, and on *mens rea*, in criminal law. Formally this would be similar to those procedures for younger individuals affected by mental diseases which are not related to brain aging.

How Can Neuroimaging Support Preventive Legislative Strategies and Criminal Law With Regard to the Elderly?

The aspect to be investigated concerns precisely the elderly individual's capacity in terms of criminal law, since due to the aforementioned reasons regarding social security, the issue requires particular attention by legislative bodies who must beforehand establish regulatory limits to the elderly individual's freedom of action and by the courts who must subsequently intervene to establish whether or not the elderly individual is guilty of having committed an illicit act. However, to date, it has been impossible to pinpoint a comprehensive strategy aimed at specifically addressing the relationship between the physiological or pathological loss of cognitive functions of the elderly and the commission of crimes.

For this purpose, the use of neuroimaging techniques would be useful to determine whether physiological or pathological aging processes have affected the individual's capacity, be it decreased or destroyed, and if there is a causal connection between the detected incapacity and the elderly individual's illicit conduct.

A desirable reform intervention must be aimed at allowing, on the preventive level, an adequate dialogic relationship between the administrative authority responsible for issuing licenses required to carry out certain risk activities and the medical staff called upon to provide the most appropriate information on the physiological or pathological brain aging of the individual seeking such licenses. Such a relationship must be founded on compliance with Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data ("General Data Protection Regulation"—GDPR), mainly with its article 9 which forbids processing of personal data concerning health, except in a number of cases among which the protection of substantial public interests is mentioned (para 2, g): the processing is licit only if it is provided for by UE or national law, proportionate to the aim pursued, respectful of the essence of the right to data protection, and accompanied by suitable and specific measures to safeguard the fundamental rights and the interests of the data subject.

To date the legislative strategies aimed at preventing harms due to the elderly performing risk activities are inadequate. In this sense, we need only to examine an authorized risk activity par excellence, namely the driving of motor vehicles: in a recent report on the relationship between road safety and elderly drivers, the European Commission found that the rate of fatal accidents involving drivers over 75 years old is five times higher than the average for drivers in general, and that this increased vulnerability is a result of the reduced physical capacity of older drivers and their decreased daily experience on the road (92). However, the European institutions have not issued to the Member States a maximum age limit for drivers concerning the grant or renewal of driving licenses (whereas in the field of commercial aircraft the European Union's strategy was more stringent, and FCL.065 of the Annex I of the Commission Regulation (EU) No 1178/2011 of 3 November 2011 established that "*The holder of a pilot licence who has attained the age of 65 years shall not act as a pilot of an aircraft engaged in commercial air transport*," due to the greater number of subjects potentially involved in a plane crash caused by senior pilots).

Among the safety measures the EU report recommended, it is important to mention the implementation of neuropsychological, medical and driving tests, aimed at establishing the ability of the elderly individual to drive and the related risks, for the purposes of granting, renewing or denying a driving license. However, it is important to point out that no reference was made to modern neuroimaging techniques which, together with the tests indicated in the report, would ensure that the physiological and pathological deficits of the brain aging process were better monitored.

Outside the European continent, a maximum age limit for the granting or renewal of driving licenses has likewise not been established. For example, in some US States the only preventive measure adopted is that of setting shorter deadlines after which elderly persons who have reached a certain age must request the renewal of their license, together with an obligation to present themselves personally before the authority for this purpose (in these cases, mail or on-line renewal is forbidden) (93). Also in this legal system no reference was made to any requirement to undergo neuroscientific tests.

In the context of prevention strategies related to those risky activities subject to licenses and without a legally established maximum age limit for their operation, the national legal systems should provide for protocols to deal with the correlations between pathological brain aging and the carrying out of risk activities that are generally permitted. Such protocols should firstly set out an obligation for more frequent and specific health checks for those who have exceeded a certain age, in order to verify the psychophysical conditions of such individuals. The combination of psychiatric and neuroscientific tests would allow authorities to establish whether the elderly individual is in MCI or a prodromal phase of dementia, where a predisposition to the development of the disease accompanied by some symptom of it may be detected. In this case, the granting or renewal of the authorization to carry out the risk activity should be subject to the adoption of certain precautionary measures. For this purpose, national legal systems need to draw up appropriate standards of diligence to regulate various risk fields, because elderly individuals might be incapable of setting the most appropriate standards of diligence or be unaware of the need to adopt such standards if they refuse to acknowledge their own deficits. In the case of driving cars, for example, it might be obligatory to have an experienced passenger next to the driver or to use vehicles equipped with safety mechanisms, such as automatic braking systems.

In order to ensure a balance between the rights of the elderly and social security, authorization must be denied in instances where the subject is in initial, intermediate, or late stage of dementia.

In the field of risk assets endangering the integrity of individuals directly in contact with the elderly in one-to-one relations (for example, the exercise of risk professions such as medical activities), the diagnosis of MCI or the prodromal phase of dementia would in itself be sufficient so as to deny the provision or renewal of authorization to carry out the activity.

With particular regard to the punishment of criminal conduct committed by individuals at an advanced age, courts will have to turn to the contribution of neuroscience, so that the assessment of the mental capacity of elderly individuals can be backed up by specialized tests indicating whether the physiological condition of cerebral aging is accompanied by pathological factors capable of prejudicing the subjects' cognitive ability. Also in this case a compliance with General Data Protection Regulation is needed, mainly with its art. 9 (para 2, f) which permits processing of personal data concerning health when it is necessary for the establishment, exercise or defense of legal claims or whenever courts are acting in their judicial capacity. In particular, diagnostic tests aimed at identifying the presence of MCI or the prodromal phase of dementia should promote widespread neuropsychiatric tests to ascertain individual mental capacity. Indeed in the US as well as in the European courts, neuroscientific evidence based on the verification of an organic or genetic predisposition to develop a specific pathology—although often considered admissible according to the Daubert criteria (94) (for example consider the decisions of the US Supreme Court in the cases of *Roper v. Simmons, Graham v. Florida*)—is not considered sufficient to overturn the presumption of capacity in force for individuals according to national and federal laws, and must be supported by traditional psychiatric tests aimed at demonstrating the existence of an actual mental disease (95). However, it is essential that neuroimaging techniques are used as evidence in criminal trials against elderly people suffering from pathological brain aging due to dementia: even after the dissemination in the US courts of the rigid M'Naughten Rules that based insanity defense on diseases pertaining only to cognitive abilities and not to those regulating self-control (will) of individuals (96) (this probative model was initially replaced by the broader and more liberal ALI test developed by the American Law Institute in the Model Penal code, and then brought back into use following the criticized absolutory outcome to which the ALI test had contributed in the famous judgement *United States v. Hinckley*), such techniques would assist judges in establishing a causal link between the perpetration of non-intentional crimes (where the volitional component is absent but the charge is based on the lack of foreseeability or failure to avoid the criminal event) and the progressive loss of cognitive functions due to the development of dementia, and thus contribute toward the application of insanity defense.

However, in chronological phases prior to the initial stage of dementia, it would be difficult to reach a non-guilty verdict by reason of insanity (NGRI), since such phases are often asymptomatic, or in any case characterized by single and asystemic episodes of cognitive deficits: thus in these stages infirmity could not be considered so intense and serious as to prove that the elderly individual lacked capacity.

Conversely the diagnosis of these early phases of the disease could lead to the elderly individuals having to deal with taking responsibility for their own lives: since it is impossible to know when exactly the first symptom will occur or at what point symptoms already present will become more numerous, the elderly persons will have to self-monitor their state (regardless of whether the administrative authority was already informed of the disease and revoked the appropriate authorization or made it subject to compliance with appropriate standards of diligence) and to refrain from engaging in risk activities or at least to undertake such activities adopting a series of precautions that would ensure adequate public safety. Failure to comply with those precautions could result in a criminal liability of the elderly for imprudently or negligently causing harm to others. In this sense we could consider that the elderly person aware of suffering from pathologic brain aging presents a kind of "culpability for assumption of a risk generally allowed," a dogmatic category used by German doctrine in criminal matters ("Ü*bernahmenfahrlässigkeit*") (97) and Italian ("*colpa per assunzione*") (98) in the context of the non-intentional responsibility of medicals for harms caused to patients and of employers for accidents in the workplace: persons who committed a crime for imprudence or negligence but were not able to foresee the harmful consequences of their action may be guilty on account of their "pre-behavior," i.e., for having undertaken a risk activity without possessing the specific cognitive skills required or alternatively for not having resorted to the special technical or informative skills of other persons who would have been able to assist them in performing the activity.

Paradoxically the previous juridical considerations regarding pathological brain aging in the phases prior to the initial development of dementia risk turning the terms of the issue at hand upside down and transforming the concept of the elderly individual from a vulnerable subject deserving of special protection to an individual responsible for having undertaken a certain risk activity and imprudently failing to consider they are gradually losing their cognitive functions.

In order to avoid a situation whereby the aforementioned paradox leads to the application of a disproportionate punishment which fails to consider the vulnerability of the elderly predisposed to develop dementia, a possible conviction of imprudent or negligent crimes for persons with advanced brain aging should be accompanied by a mitigation of the penalties imposed (99). This could be done either by applying a generic extenuating circumstance or by introducing an appropriate mitigation based on an evaluation of the regression of cognitive functions in the elderly individual, in parallel with the provisions established in some legal systems for accused minors (see art. 98 of the Italian Criminal Code). For example such a mitigation was provided for by art. 75 of Venezuelan Criminal Code which established that individuals over 70 years of age who have been convicted of a crime are not punishable by presidio or imprisonment but only by arrest not exceeding 4 years. Lastly it would also be auspicious to examine whether conditions regarding appeals for alternatives to detention (such as probation) might be simplified (100), so as to avoid elderly persons having to enter into a prison environment which would be likely to worsen their physical and mental health and whose capacity for rehabilitation would be severely affected by their own old age (101).

In cases where a prison sentence cannot be avoided (intentional offenses of major disvalence), it would also be advisable to establish special courts and special prison sections for the elderly so that their contact with the justice system is not traumatic and that they can take advantage of special re-education and resocialization programs (102). In this particular prison environment, elderly individuals could be in contact and socialize with prisoners of their same age in order to have a greater chance of integration and socialization. Constructive meetings and dialogues with family members and younger people could be guaranteed by a mechanism which could provide more frequent visits of external visitors to the prison together with an appropriate number of temporary release permits for prisoners and lastly by ensuring a valid support network of psychologists and social workers.

CONCLUSIONS

Knowledge of the multidimensional process of brain aging and of the factors that influence its evolution on the clinical and behavioral level is of fundamental importance for its correct and global management. The control and treatment of vascular risk factors and endocrine disorders can reduce the prevalence of long-term disability in the elderly population. Technological advances in brain imaging help us, whether it be in daily practice, in research protocols or in pharmaceutical trials, to improve the quality of aging, to increase the number of individuals that age successfully, and to slow down and control the processes of pathological aging. However, the responsibility of clinical practitioners in the detecting of diseases and in the management of informations must not be forgotten, especially in presence of pathological brain aging suitable to affect diligent behaviors of elderly in risky assets where public security may be endangered: for this purpose, the use of brain imaging and the cooperation with practitioners have to become usual for the legislator, aimed at setting up a prevention strategy, and for the courts, in order to ascertain guiltiness of elderly individuals involved in criminal acts.

AUTHOR CONTRIBUTIONS

VT conceived the review and wrote the legal paragraphs of the manuscript. GLC collected the images and references and wrote the manuscript. CS-C and PP critically revised the manuscript. US wrote and critically revised the manuscript for important intellectual content.

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REVIEW
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Androgen Deficiency and Phosphodiesterase Type 5 Expression Changes in Aging Male: Therapeutic Implications

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The age-related decline of serum T occurs in ~20–30% of adult men and it is today defined as late-onset hypogonadism (LOH). In the elderly, such decline becomes more prevalent (up to 60%) and shows-up with erectile dysfunction

(ED) and hypoactive sexual desire. A large body of experimental evidences have shown that the combination of T replacement therapy (TRT) and phosphodiesterase type 5 inhibitors (PDE5i) is, usually, effective in restoring erectile function in patients with LOH and ED who have not responded to monotherapy for sexual disturbances. In fact, PDE5is potentiate the action of nitric oxide (NO) produced by endothelial cells, resulting in a vasodilator effect, while T facilitates PDE5i effects by increasing the expression of PDE5 in corpora cavernosa. Meta-analytic data have recognized to PDE5i a protective role on the cardiovascular health in patients with decreased left ventricular ejection fraction. In addition, several studies have shown pleiotropic beneficial effects of these drugs throughout the body (i.e., on bones, urogenital tract and cerebral, metabolic, and cardiovascular levels). TRT itself is able to decrease endothelial dysfunction, oxidative stress and inflammation, thus lowering the cardiovascular risk. Furthermore, untreated hypogonadism could be the cause of PDE5i ineffectiveness especially in the elderly. For these reasons, aging men complaining ED who have LOH should undergo TRT before or at the moment when PDE5i treatment is started.

Keywords: aging, hypogonadism, erectile dysfunction, sexual desire, pde5 expression, pde5 inhibitors, testosterone replacement therapy, elderly

INTRODUCTION

Male hypogonadism is generally characterized by abnormally low serum T (T) levels. Cross-sectional studies have found that 20–64% of old men with diabetes have hypogonadism, with higher prevalence rates found in the elderly. Typical symptoms include sexual dysfunctions, changes in mood, decreased bone mineral density, increased body fat and decreased muscle mass and strength (1). By restoring serum T levels to the normal range using T replacement therapy (TRT), many of these symptoms can be relieved.

A number of other common conditions can also be associated with decreased T production in the elderly. These include metabolic syndrome (MetS), atherosclerosis, myocardial infarction, and chronic heart failure (2). Several studies have shown an increased cardiovascular disease (CVD) risk of up to 4-fold in men with either MetS or type 2 diabetes (3–5). Studies have also shown that low T levels in men can predict the development of insulin resistance, the physio-pathological basis of MetS, and a possible progression to type 2 diabetes (6, 7). Men are twice as likely as women to develop CVD as well as diabetes. This might be ascribe to differences in endogenous sex hormone levels. Indeed, patients with type 2 diabetes mellitus have lower androgen levels and poorer glucose tolerance than non-diabetics (8–10). Thus, low serum T levels are associated with an increase in many of the known cardiovascular risk factors (11) listed in this Table 1.

Table 1. Biochemical and metabolic effects of T (T) deficiency and their reversal after T replacement therapy (TRT).

Low T			TRT
HDL cholesterol	↓	↓	(Smaller ↓ observed in older men)
Total cholesterol	↑	↓	Total cholesterol
LDL cholesterol	↑	↓	LDL cholesterol
Triglycerides	↑	↓	Apoprotein B
		↓	Lipoprotein a
Hypertension	↑	↓	Diastolic BP by 4–5 mmHg
Fibrinogen	↑	↓	Fibrinogen
PAI-1	↑	↓	PAI-1
Visceral obesity	↑	↓	Visceral obesity
Fasting glucose	↑	↑	Insulin sensitivity
Fasting insulin	↑	↓	Insulin resistance

Adequate T concentrations are also crucial for a proper endothelial function, for the expression of penile PDE5 isoenzyme (12) as well as for the adequate production of hydrogen sulphyde (H_2S). Thus, long-term TRT would be expected to decrease cardiovascular morbidity and mortality but is not recommended in the frail elderly (13). Men with ED and low T levels are potential candidates to benefit from a combination therapy if response to monotherapy is not sufficient. A combined treatment may result in endothelial rejuvenation by potential remodeling of vascular wall (14). However, since the potential high benefits from these therapies in specific elderly population have not yet been proven, the purpose of this article is to review basic and translational experimental evidences that support a possible role of T in the regulation of PDE5 expression in the urogenital tract and to evaluate its use, alone or in combination for the treatment of patients with LOH and sexual dysfunctions.

ROLE OF CYCLIC NUCLEOTIDES AND PDES IN T PRODUCTION AND PENILE ERECTION

In Leydig cells (LCs), the production of T is regulated by the cyclic adenosine monophosphate (cAMP) signaling pathway. Luteinizing hormone (LH) binds to its receptors coupled to the G-protein that regulates adenylyl cyclase (ADCYs). This event leads to an increase in the intracellular cAMP levels with subsequent activation of protein kinase A (PKA) that promotes steroidogenesis (15).

The cyclic guanosine monophosphate (cGMP) signaling pathway is also active in LCs and, together with the cAMP signaling pathway, modulates steroidogenesis in LC (16, 17). In these cells, nitric oxide (NO), generated by NO synthases endothelial (eNOS) and/or inducible NO synthase (iNOS), stimulates the production of cGMP. The cGMP, in turn, activates the protein kinase G (PKG) that phosphorylates the acute regulatory steroidogenic protein (StAR), thus promoting steroidogenesis (17, 18).

An inverse relationship between NO production and T secretion has been shown (19). Subsequently, a biphasic relationship was described. Valenti et al. reported that higher concentrations of NO donors decrease T production whereas lower concentrations increase its levels (20). This occurs because at lower concentrations NO activates cGMP-dependent pathway leading to the activation of PKG-1 and consequently the phosphorylation of StAR protein that promotes steroidogenesis (17). Conversely, at higher concentrations, NO directly inhibits the activities of steroidogenic enzymes in LCs (19, 21).

An interaction between the nitrergic and purinergic systems seems to exist in LCs. In fact, recently, it has been shown that basal NO production in LCs changes the adenosine triphosphate (ATP)-evoked currents and that extra NO modulates the current through a mechanism involving the NO/cGMP signaling pathway (22).

The spatiotemporal dynamics of cAMP and cGMP pathways depends upon PDE activity, which by breaking phosphodiesteric bonds terminate cyclic nucleotides signaling (23). In mammals, 11 PDE families exist. These include: PDE4, PDE7, and PDE8 are highly specific for cAMP; PDE5, PDE6, and PDE9 are highly selective for cGMP; while PDE1, PDE2, PDE3, and PDE10 act on both molecules (23).

PDE5A, a cGMP-specific PDE, is expressed in LCs (24) where it seems to modulate cGMP/PKG-stimulated androgen production, as shown by the raise in cGMP and androgen levels after treatment with a selective PDE5i (17). In LCs, T production is also suppressed by the activity of PDE8A, an enzyme that specifically hydrolyzes cAMP. In fact, LCs from PDE8A-null mice secrete about 4-fold more T compared to those of wild-type mice and are more responsive to LH stimulation (25). These data indicate that both cAMP and cGMP are involved in T production, and that PDEs contribute to the regulation of androgen synthesis in LCs and could be target of pharmacological manipulation.

cGMP and cAMP are also fundamental in the regulation of the vascular processes that lead to erection. Endothelial cells produce NO, which in turn activates soluble guanylyl cyclase (sGC). The subsequent accumulation of cGMP induces the relaxation of smooth muscle in corpora cavernosa (26). cAMP contributes to erection physiology through the cyclooxygenase-2 (COX-2) pathway. COX-2 and prostacyclin synthase (PTGIS) catalyze the synthesis of prostaglandin E which, by binding to specific receptors on smooth muscle, activates cAMP-dependent pathways that lead to muscular relaxation (27). The main penile regulatory biochemical machinery is summarized in Figure 1.

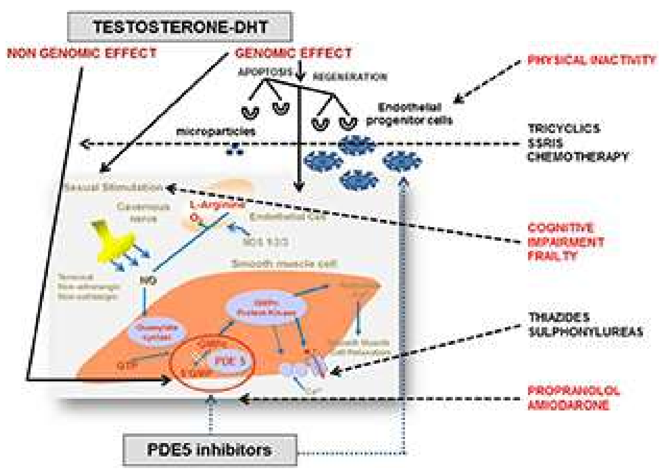

FIGURE 1. Endocrine and pharmacological regulation of penile erection. In corpora cavernosa the effects of testosterone (T) are primarily mediated by its conversion into 5α-dihydrotestosterone (DHT) and its binding to the androgen receptors (ARs), localized within vascular endothelium and smooth muscle cells. The AR is a ligand-activated transcription factor acting on the genome. The genomic action of AR is modulated by a large variety of co-regulators, that fine-tune target gene expression by enhancing or restraining transcription. However, steroids, including androgens, have also membrane receptors responsible for non-genomic actions. The non-genomic T pathway involves the rapid induction of conventional second messenger signal transduction cascades (i.e., activation of protein kinase A, protein kinase C, and MAPK), leading to diverse cellular effects including smooth muscle relaxation, neuromuscular and junctional signal transmission, and neuronal plasticity. Nitric oxide (NO) is released from nitrergic nerve endings and from the endothelium in response to acetylcholine and to the shear stress due to increased blood flow. Subsequently, NO penetrates into smooth muscle cells and binds to soluble guanylate cyclases, which catalyze the conversion of

guanosine triphosphate to cyclic guanosine monophosphate (cGMP). cGMP activates protein kinase G which phosphorylates and activates proteins that reduce the intracellular Ca^{2+} concentration or the sensitivity to Ca^{2+}, decreasing muscular tone. Phosphodiesterase type 5 (PDE5) is the predominant enzyme responsible for cGMP hydrolysis in vascular and trabecular smooth muscle. T regulates PDE5 expression and mediates clinical response to PDE5 inhibitors through a putative androgen response element (ARE) in human PDE5A1 promoter. Many interfering factors in the elderly (i.e., frailty, physical inactivity, and polypharmacy) may directly or indirectly block at multiple site the physiological pathways. Adapted from Aversa et al. (28). NOS, nitric oxide synthase; GMP, guanosine monophosphate; GTP, guanosine triphosphate; SSRI, selective serotonin reuptake inhibitors.

AGE-RELATED CHANGES IN T PRODUCTION AND PDE EXPRESSION IN THE UROGENITAL TRACT

It has been shown that serum T levels decrease ~by 1% per year in men from their 30th (29). This is in part due to a progressive age-related decline in T production by LCs. The main causes of this decrease include: a diminished response of cAMP to gonadotropins; a lower LHR expression in LCs; a decline in StAR transcription with consequent impairment of cholesterol intracellular availability; and a decreased activity of some steroidogenic enzymes (30, 31). Another mechanism is the GnRH signaling attenuation in aging men, due to decreased GnRH gene expression and altered pulsatility and amplitude of GnRH pulse (32).

LC of aged rats have lower cAMP concentration whereas NO–cGMP signaling is increased (33). Sokanovic et al. have shown a progressive increase in endogenous NO production in aged rats that contributes to the decrease in T production with a mechanism independent from the cGMP pathway (33). The increase in cGMP alone improves T content in LCs of both adult and aged rats, while an increase in NO levels enhances cGMP and inhibits cAMP production, with consequent T production decrease, but only in LCs from aged rats (33). These findings show that cGMP stimulates and NO inhibits steroidogenesis in aged rats.

Aged animals not only have significantly lower T concentration than adults but they also loose the normal T secretion rhythmicity (31). In LCs of aged rats, the alteration also occurs in the transcription rhythmicity of genes involved in both cAMP and cGMP pathways (34). Aging has been suggested to strengthen the negative cross-talk between the two signaling pathways through changes in the expression of PDEs with dual activity, but these data have to be confirmed (34).

Furthermore, PDE5 gene is over-expressed in LCs of aged rats and its increased activity has been associated with lower T production. Data are less clear in endothelial cells of corpora cavernosa. To our knowledge, data on age-related PDE5 gene expression changes in the corpora cavernosa are not available. Indirect information comes from studies that investigated the effects of hypogonadism and TRT on PDE5 gene expression. Lower T levels are one of the features of aging but it is not always present in elderly men and, furthermore, other mechanisms, independent from T, could be involved in the regulation of the expression of PDE genes in the elderly. Up-regulation of PDE5 gene is believed to be one of the mechanisms underlying androgen therapeutic effects in the treatment of ED (35–37). This belief derives from the finding of a putative presence of the androgen response element (ARE) in the human PDE5A1 gene promoter (38). However, more recently, the same authors have criticized the results of their previous studies. In fact, the up-regulation of the PDE5 gene by T would create a paradox in which a positive regulator of erectile function (androgen) would increase the level of a negative regulator (PDE5), potentially leading to worsening of ED and to a more difficult clinical management (39). Moreover, if so, in the corpora cavernosa would occur the exact opposite of what happens in LCs, where aging-related T decrease is associated to an increased expression of PDE5 (34). Finally, two studies that have looked for androgen-responsive genes in the whole human genome and they found respectively 524 and 1,532 potential AR-binding sites, but PDE5A gene was not among them (40, 41). Therefore, further studies are needed to clarify the relationship between androgens and PDE5 gene expression in the corpus cavernosum, but it is well-established that TRT improves the effect of PDE5i treatment in patients with hypogonadism (see Synergic Effect of T Plus PDE5is in the Treatment of Erectile Dysfunction in Patients with LOH).

cGMP pathway also plays a fundamental role in bladder, prostate, seminal vesicle and epididymis physiology. In these organs, the cGMP-signaling pathway regulates muscle contractions and peristalsis, cell proliferation, and secretory activity (42). The urogenital organ with the most active cGMP signaling pathway seems to be the bladder, where NO–cGMP pathway regulates the micturition reflex and the phasic contractile activity (43, 44). A study revealed that bladder shows high expression of PDE5 and that the amount of this protein is significantly lower in aged bladder than in younger ones, probably due to the age-related decrease in muscular content (42). These findings remark the pivotal role played by cGMP pathway in the physiology of the bladder, which could therefore represent a favorable target for PDE5i pharmacological action (42).

With aging, prostate progressively develops benign prostatic hyperplasia (BPH), that is present in up to 90% of men over 80 years of age (45). BPH is characterized by enlargement and alteration of stromal compartment, focal proliferation of smooth muscle cells, epithelial basal cell hyperplasia, and nodular arrangement of the transition zone of the gland (46). Development of BPH is multifactorial; one of the pathophysiological mechanisms involves the increase in estrogen/androgen ratio in prostatic stromal tissue, due to the lower T production and conversion to DHT. Other factors are the interaction between growth factors (IGF, FGF, TGF) and steroid hormones and chronic prostate inflammation (47). A study has shown that prostate is an organ with poor expression of all enzymes implicated in cGMP signaling pathways, including PDE5; but PKG1 expression shows an age-related increase in rat prostate cells (42). In the same study, Authors, considering the pronounced androgen dependency of prostate, investigated the expression of cGMP pathway proteins in this tissue in conditions of androgen deprivation. Interestingly, they showed a further upregulation of PKG1 and a less pronounced increase in PDE5 expression (42), similarly to what Baburski et al. showed in LCs of aged rats (34). At the prostate level, the cGMP pathway could be implicated in the relaxing activity and in the regulation of proliferation and differentiation of smooth muscle cells. In fact, PDE5is showed the ability to lower the proliferation of prostate stromal cells and fibroblast-to-myofibroblast trans-differentiation (48). Authors, therefore, speculated that PKG1 could be directly implicated in cellular proliferation processes: decreased androgen levels could increase prostatic PKG1 expression and, in turn, promote cell proliferation (42). Another study has shown an up-regulation of PDE5 in both rat and human BPH, which was immunolocalized in prostate fibromuscular stroma. Since BPH was obtained in experimental models by T administration, the authors speculated that the increased PDE5 expression could be due to the increase in T levels (49), partly contradicting the results of Müller et al. that showed an increase in PDE5 expression following T deprivation. BPH is an androgen-dependent disease. In fact, androgen ablation (by administration of GnRH agonists, androgen receptor antagonists or DHT inhibitors) is an effective strategy in decreasing prostate volume. We also speculate that these beneficial effects may be mediated by a decreased expression of PDE5, which is androgen-dependent in the rat bladder, and therefore by an enhancement of NO-induced relaxation during the filling phase. This latter aspect may account for the beneficial effect of daily PDE5i use on detrusor overactivity (50). Nevertheless, further investigations are needed to clarify the mechanism that regulate PDE5 expression in prostate cells.

LATE-ONSET HYPOGONADISM AND ERECTILE DYSFUNCTION

The age-related T decline, known in the past as male menopause or andropause, is today defined as late-onset hypogonadism (LOH) (29, 32). Hypogonadism is diagnosed when at least two T measurements, obtained from morning blood samples, are low in the presence of signs and symptoms of androgen deficiency (51). The most specific symptoms associated with LOH are the sexual ones: decreased frequency of morning erection, decreased frequency of sexual thoughts, and ED (52).

ED in hypogonadal patients is strictly related to systemic endothelial dysfunction. In fact, T is able to promote angiogenesis and endothelial cell proliferation through a mechanism mediated by the androgen receptor (AR)/vascular endothelial growth factor (VEGF) pathway (53). Endothelial microparticles (EMPs) are fragments of the plasma membrane released from the injured vessels and are considered a marker of endothelial dysfunction. Their concentration increases in patients with LOH and ED (54). Endothelial progenitor cells (EPCs) are a group of cells, similar to the embryonic angioblasts, that can originate from the mesoderm or from transdifferentiated monocyte/macrophages. EPCs could have different possible phenotypes and are implicated in the vasculogenic reparative process and the consequent re-endothelization after vascular injuries (54). Some EPC populations are decreased in hypogonadal patients (55), while other EPCs subtypes are present in higher concentration in patients with hypogonadism and ED, compared to eugonadal patients with ED (56). This last subpopulation of EPCs (i.e., EPCs CD45neg/CD34pos/CD144pos) could be considered a marker of vascular damage, in response to which they are produced in greater quantities in the attempt to repair the injured endothelium. In fact, they have been found in higher concentrations in the blood of patients with coronary artery disease, and their levels increase as ED worsens (54, 57). Furthermore, it has been shown that a greater endothelial damage is related to a worse pharmacological response to PDE5i (58).

The oxidative stress is another mechanism by which hypogonadism affects endothelial function. In fact, it has been shown that hypogonadism increases oxidative stress and decreases NO bioavailability (59–61). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is an enzymatic complex that catalyzes the production of reactive oxygen species (ROS) (62). In castrated rats some NADPH oxidase subunits are over-expressed and this up-regulation leads to increased ROS production in the corpora cavernosa (63). In this model, TRT lowers the expression of NADPH oxidase and, consequently, ROS production, increases NO bioavailability and improves erectile function (63). In the same study, the Authors showed that hypogonadism decreased the expression of COX-2 and PTGIS, leading to a decreased penile cAMP levels. TRT also restores COX-2 and PTGIS expression, and increases cAMP concentration (63). Therefore, the decrease of ROS production and the activation of COX-2/PTGIS/cAMP signaling pathway with consequent increase in cAMP production might represent two mechanisms through which TRT may restore erectile function in hypogonadal patients.

Hypogonadism is associated with a low-grade inflammation that may be involved in the pathogenesis of androgen deficiency symptoms in aging men. A correlation between C-reactive protein (CRP) and aging male symptom (AMS) score has been reported, and a reduction in CRP levels and AMS scores was shown following TRT (64). The concentrations of IL-6, NF-Kb mRNA, and asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor that increases in response to inflammation, have been shown to be higher in castrated rats compared to controls (65). T administration to castrated rat decreases these markers of inflammation suggesting that T deficiency could increase oxidative stress and endothelial dysfunction by stimulating inflammation (65).

The endothelial dysfunction in hypogonadism is a systemic event, not exclusively confined to the penile district. Several epidemiological and observational studies have shown that low T levels are associated with cardiovascular diseases as atherosclerosis, coronary artery disease, and coronary events (66). A meta-analysis of 70 studies found significantly lower T levels in patients with cardiovascular diseases than controls (67). Finally, ED itself, one of the main symptoms of hypogonadism, is an independent risk factor for cardiovascular disease and it predicts the presence and the extent of subclinical atherosclerosis (68). In the aging male with LOH, the endothelial damage related to hypogonadism is added to the age-related endothelial dysfunction due to an imbalance between oxidative stress and antioxidant status, which predisposes elderly patients to cardiovascular events (69).

It is noteworthy that systemic endothelial dysfunction and atherosclerosis can also affect the microcirculation of the testis and cause a LC dysregulation with consequent lower T production (70). Therefore, a vicious circle could be established: in the elderly, LOH worsen age-related endothelial dysfunction that leads to a further T production decrease by affecting testicular microcirculation.

SYNERGIC EFFECTS OF T PLUS PDE5IS IN THE TREATMENT OF ERECTILE DYSFUNCTION IN PATIENTS WITH LOH

PDE5is are the first choice drugs for the medical treatment of ED (71, 72). PDE5is inhibit the effect of PDE5, which terminates cGMP's effects breaking down its phosphodiester bond. The consequent intracellular accumulation of cGMP activates cGMP-dependent protein kinase which phosphorylates specific proteins implicated in a number of physiological responses, such as smooth muscle relaxation, platelet aggregation, and cardiac functions (73). In corpora cavernosa, NO is released from nitrergic nerve endings, from the endothelium in response to acetylcholine released by parasympathetic endothelial nerve endings, and by the shear stress due to increased blood flow in the sinusoids. NO penetrates into smooth muscle cells and binds to sGC, which catalyze the conversion of guanosine triphosphate to cGMP. cGMP activates PKG which phosphorylates and activates proteins that reduce the intracellular Ca^{2+} concentration or the sensitivity to Ca^{2+}, decreasing, consequently, the muscular tone (73). The final result is vasodilatation and an increased blood flow into the cavernosal sinusoids which leads to erection. PDE5is potentiate this effect by increasing the level of intracellular cGMP when the NO-signaling pathway is activated.

PDE5is currently available (sildenafil, vardenafil, tadalafil, avanafil, mirodenafil, udenafil, and lodenafil) show the same pharmacodynamics, but they differ from each other for the pharmacokinetic properties. Avanafil and vardenafil have the quickest onset of action, whereas tadalafil shows the longest half-life (up to 36 h) (72). This favorable pharmacokinetic feature allowed to approve tadalafil for daily use, while the other PDE5is are usually administrated on-demand.

About 30% of patients are poor responders to PDE5is (74). One of the causes that can impair the response to PDE5i is indeed hypogonadism (75). In the corpora cavernosa of experimental models, the androgen deprivation causes smooth muscle cell apoptosis and adipose tissue deposition with consequent fibrosis; decreased expression of eNOS and neuronal nitric oxide synthases; decreased arterial inflow and increased venous outflow; enhanced response to vasoconstrictor mediators such as α-adrenergic agents; and decreased NO-mediated smooth muscle relaxation after sexual stimulation (75).

Another modification ascribed to hypogonadism is a decreased PDE5 expression (see Figure 1). Some studies did not confirm the presence of an ARE in the human PDE5A gene promoter initially described (39), but several studies anyway reported a down regulation of PDE5 expression in the corpora cavernosa, prostate and bladder in animal models after surgical or pharmacological castration. In all these studies, PDE5 expression was restored by T administration (12, 76–78). It has been reported that castration lowers the content of smooth muscle cells in the corpus cavernosum and prostate, which are replaced by non-muscular cells such as adipocytes (79, 80). PDE5 is expressed in smooth muscle cells of corpora cavernosa, resulting in a lower amount of substrate on which PDE5is may act, making less effective the action of these drugs. Thus, TRT could facilitate the pharmacological effects of PDE5is, restoring the structure of the corpora cavernosa and increasing their content in smooth muscle cells and, consequently, the expression of PDE5 (39). Furthermore, this would explain the temporal interval (up to 6 months) necessary for T to improve erectile function, as the structural modifications of the corpora cavernosa induced by TRT require time to be completed (81).

TRT has also been proven effective in lowering circulating EPC and EMP concentrations and in restoring NO levels in corpora cavernosa (82–84). This suggests that TRT improves erectile function by decreasing the degree of endothelial damage. A similar effect seems to be produced by physical activity. Indeed, a recent study showed that in patients with LOH a protocol of 150 min per week of moderate-intensity aerobic exercise in association with tadalafil 5 mg daily for 90 days improves erectile function even if total blood T levels are below normal. The improvement of erectile function was shown by an increased international index of erectile function 5 (IIEF5) score and the main vascular arterial parameters (acceleration time and peak systolic velocity) evaluated by penile Doppler ultrasound (85).

Increased efficacy of combined T and PDE5i administration compared to PDE5 monotherapy in hypogonadal patients has been shown by several studies (86–93). Furthermore, the administration of T undecanoate plus once-daily tadalafil 5 mg is more effective in restoring erectile function than the combination of T undecanoate plus on-demand tadalafil 10–20 mg. Over 30 weeks of treatment, patients treated with T undecanoate plus once-daily tadalafil 5 mg showed higher IIEF5 and AMS scores and, after 6 weeks from treatment discontinuation, a higher percentage of patients had a maintenance of their subjective erectile function improvement (94).

The synergic effect of T plus PDE5i seems to be evident also when patients begin TRT at first (Figure 2). Yassin et al. showed that just under 50% of hypogonadal patients with ED fail to respond to T undecanoate treatment alone within 3 months. Almost all of these patients respond well to the addition of 20 mg vardenafil on demand (97). In another study, hypogonadal patients received 1% T gel and 100 mg sildenafil was added to those who did not obtain an improvement in erectile function after 3 months of therapy. All these patients responded well to the combination therapy (98). A bias of these two studies may be the duration of TRT before the addition of PDE5is, because in some patients (those with more severe or long-standing hypogonadism), the therapeutic efficacy of TRT may become evident after more than 12 weeks (81).

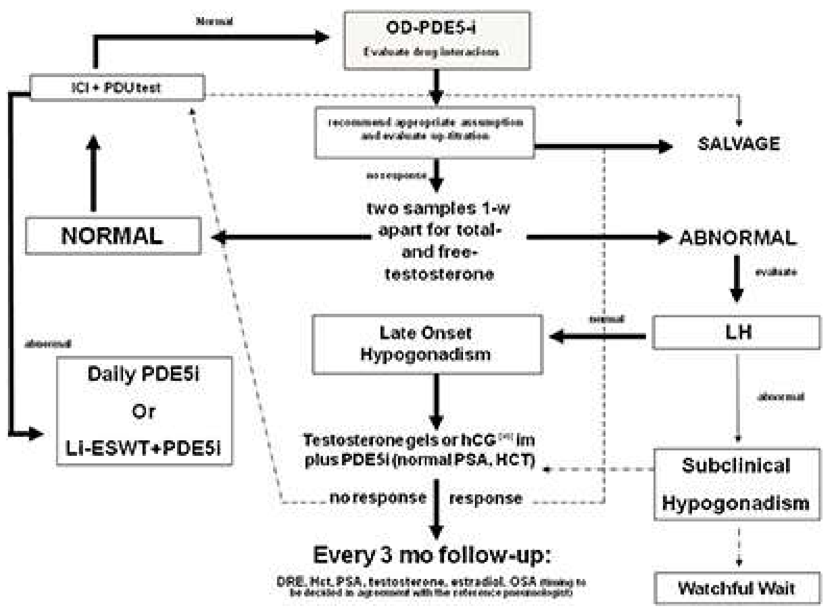

FIGURE 2. Diagnostic flow-chart for the elderly patient with sexual dysfunction not responding to oral PDE5i. Testosterone (T) deficiency could be a cause of non-response to treatment with PDE5i. Therefore, in patients with hypogonadism any treatment for erectile dysfunction (ED) should be initiated with T, whereas PDE5is could be co-administered for immediate relief of poor erection complaints. In the elderly, T levels must be preferably restored by the administration of T in gel formulation. Patients with normal gonadotropin levels could also benefit by hCG administration. Follow-up must be performed every 3 months evaluating PSA, blood count, and androgen levels. A digital rectal examination and a pneumological evaluation could also be indicated. In patients with normal blood T levels, the prescription of PDE5is alone on demand is always recommended. If the patient does not respond to this treatment, the second step is to evaluate any drug interaction, the correct PDE5i intake, and the eventual drug up-titration. Penile pharmaco-testing with alprostadil (ICI) followed by penile dynamic Duplex ultrasound (PDU) may retrieve important data regarding vascular health and stratification of cardiovascular risk as well as possible therapeutic approaches (96). These include daily PDE5is, intra-cavernous or intra-urethral alprostadil administration, and low-intensity extracorporeal shockwave therapy [adapted from Aversa et al. (75)]. OD, on demand; DRE, digital rectal examination; PSA, prostate specific antigen; Li-ESWT, low-intensity extracorporeal shockwave therapy; OSA, obstructive sleep apnea.

Recent evidence showed that PDE5is, until recently considered a symptomatic therapy for ED, can partly exert an effect on the pathophysiological mechanisms that lead to LOH. Sokanovic et al. treated aged rats with oral sildenafil, a specific inhibitor of PDE5, and, after 3 and 6 months of therapy, they found an improvement in steroidogenesis. The Authors showed an increase in cGMP/NO ratio, a decreased serum nitrite levels, and an increased cAMP content of LCs (33). They also analyzed PDE gene expression in LCs from sildenafil-treated animals and controls. Aging produced alterations in the pattern of PDE gene expression and treatment with PDE5is was able to reverse these alterations, contributing to the normalization of cAMP levels. Finally, sildenafil treatment increased the transcription of key genes for steroidogenesis (CYP11a, CYP17a1, HSD3b, HSD17b4, and StAR) probably contributing to the increase in androgen levels found in aged rats treated with sildenafil (33). In a recent study, the same group reported that long-term PDE5 inhibition slows-down the regressive changes that take place in testes during aging (99). Similar results have also been reported in humans. For example, Spitzer et al. found that sildenafil administration in patients with ED and low serum T levels leads to a significant increase in total and free T and a decreased serum LH concentration, suggesting a direct effect of PDE5is at the testicular level (100).

Overall, these data indicate that T and PDE5i act synergistically to improve erectile function in patients with LOH. Chronic androgen deprivation leads to anatomical and histological alterations of the corpora cavernosa that make the pharmacological action of PDE5is sometimes ineffective (see diagnostic algorithm in Figure 2). For this reason, it is more appropriate to normalize T levels before starting PDE5i administration, taking into account that the structural improvement of the corpora cavernosa and, consequently, of the erection can take up to 6 months to occur (81). In parallel, PDE5is have been shown to improve T levels in patients with LOH and, in animal models, to prevent or slow-down the regressive changes, partially responsible for the onset of LOH, that occur in the testis during aging. Therefore, PDE5i therapy could prevent aging-related testicular alterations (and then LOH) and in clinical hypogonadism may be effective in normalizing androgen levels in association with TRT.

CONCLUSIONS

Aging leads to a progressive decrease in androgen production that, in turn, leads to the development of LOH, defined by significant low T serum levels (in the lowest quartile) in the presence of signs and symptoms of hypogonadism (51). LOH could be due to both testicular and hypothalamic-pituitary dysfunction (32), and ED is one of its main symptoms. ED in LOH is linked to increased oxidative stress, subclinical inflammation, and subsequent endothelial dysfunction (101). In elderly men, it has been shown that LOH is also linked to lower cAMP pool and to an alteration of the cGMP signaling pathway.

PDE5 gene lower expression is associated to aging and hypogonadism at the corpus cavernosum level. TRT is able to restore the expression of PDE5 gene and this effect is initially attributed to a direct regulation of the gene expression by T (38). Subsequently, this hypothesis was not confirmed, and the authors hypothesized that the lower expression of PDE5 in hypogonadism was due to the decreased smooth muscle cell content in corpora cavernosa. Therefore, T could able to increase PDE5 content by reversing these anatomical changes (39). Anyway, the increased PDE5 gene expression explains the reason for the possible failure of PDE5i administration in hypogonadal patients with ED.

The timing of treatment with T and PDE5is in patients with hypogonadism and ED is a matter of debate (102). The initial approach to patients with ED encompasses the use of PDE5is (72) (Figure 2). However, as we have seen, hypogonadism is, especially in aging men, a common cause of ED and a reason for a lack of response to PDE5is (71). Hence, patients with ED should be tested for androgen deficiency before treatment with PDE5i is given (102), because TRT it is effective in about half of the patients with ED (84). The addition of PDE5is should be reserved to those patients in whom ED persists despite the eugonadal state restoration. However, the time-course of T effects requires long-term administration to become detectable (81).

PDE5is showed the ability to enhance steroidogenesis at the testicular level, to reverse the age-related alterations of PDE genes expression (33, 100), and to slow-down age-related regressive alteration of the testis (33). Furthermore, PDE5is have pleiotropic actions throughout the body that could counteract the age-related physiopathological alterations that affect the urological tract and male accessory sexual glands (48, 103), bone (104), fat tissue (105), brain (106), and heart (107).

Before starting any treatment, elderly men should be accurately investigated for the presence of major contraindications to the use of TRT and/or PDE5is even in the presence of hypogonadism. Once this work-up is completed, treatment(s) should be wisely offered to improve their sexual function whenever cardiovascular efficiency is proven.

From a clinical-translational point of view, the information provided in this review would suggest careful consideration of the systemic implications of hypogonadism in the elderly and the benefits of treatment since there is disagreement on the threshold value for its safe prescription. In summary, we suggest a total T value <8 nmol/L along with uncompensated LH levels and relevant clinical symptoms i.e., sexual symptoms, sarcopenia, anemia, osteoporosis (12–14, 36, 37, 50, 75, 81).

Several studies have shown an inverse relationship between indicators of obesity (body mass index, waist circumference, a reliable indicator of visceral obesity), DM2/metabolic syndrome and T levels over all age groups. Hence, erectile dysfunction may be considered a predictor of severe peripheral vascular damage when compared to healthy population and should be regarded as a major health threaten for the older patients (47, 54, 57, 58, 83, 85, 101, 103).

AUTHOR CONTRIBUTIONS

AA and SL conceived the idea and revised the manuscript. YD wrote the manuscript. AC and RC performed the literature search, and corrected syntax and typos.

ABBREVIATIONS

ADCY, adenylyl cyclase; ADMA, asymmetric dimethylarginine; AMS, Aging Male Symptom; AR, androgen receptor; ARE, androgen-response element; ATP, adenosine triphosphate; BPH, benign prostatic hyperplasia; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; COX-2, cyclooxygenase-2; CRP, C-reactive protein; CVD, cardiovascular diseases; ED, erectile dysfunction; EMPs, endothelial microparticles; eNOS, endothelial nitric oxide synthases; EPC, endothelial progenitor cells; GnRH, gonadotropin-releasing hormone; H_2S, hydrogen sulphyde; IIEF5, International Index of Erectile Function 5; iNOS, inducible nitric oxide synthases; LCs, Leydig cells; LH, luteinizing hormone; LHR, luteinizing hormone receptor; MetS, metabolic syndrome; NADPH, nicotinamideadenine dinucleotue phosphate; nNOS, neuronal nitric oxide synthases; NO, nitric oxide; PDE, phosphodiesterase; PDE5i, phosphodiesterase 5 inhibitors; PKA, protein kinase A; PKG, protein kinase G; PTGIS, prostacyclin synthase; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; StAR protein, steroidogenic acute regulatory protein; T, T; TRT, T replacement therapy; VEGF, vascular endothelial growth factor.

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REVIEW
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Hypothyroidism as a Predictor of Surgical Outcomes in the Elderly

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There is a high prevalence of hypothyroidism in the elderly population, mainly among women. The most important cause is autoimmune thyroiditis, but also iodine deficiency, radioiodine ablation, and surgery may be responsible for hypothyroidism in elderly hospitalized patients. Thyroid-related symptoms are sometimes comparable to physiological manifestations of the aging process, and hypothyroidism may be related with many symptoms which can be present in critical patients, such as cognitive impairment, cardiovascular, gastrointestinal, and hematological alterations, and eventually myxedema coma which is a severe and life-threatening condition in older adults. Adequate thyroid hormone levels are required to achieve optimal outcomes from any kind of surgical intervention. However, only few randomized clinical trials investigated the association between non-thyroidal illness (or low-T3 syndrome), and adverse surgical outcomes, so far. The goal of this review is to discuss the role of thyroid function as a predictor of surgical outcomes in the elderly.

Keywords: hypothyroidism, elderly, surgery, thyrotoxicosis, low T3 syndrome

KEY CONCEPTS

- The achievement of euthyroidism represents the goal before elective surgery, in order to prevent the risk of complications. In non-elective surgery, a careful risk-benefit evaluation in hypothyroid patients before surgical treatment is needed.
- The range of thyroid hormone levels in older patients may be different compared to that in younger subjects. Features of physiological aging may be occasionally confused with hypothyroidism in elderly patients.
- An adequate titration of LT4 in older patients is mandatory to attain appropriate serum TSH concentrations and avoid the risk of iatrogenic thyrotoxicosis.

INTRODUCTION

Primary hypothyroidism is the most frequent pathological hormone insufficiency; its prevalence is approximately 10 times higher in women compared to men, and its incidence raises with age (1) (Table 1). The UK Whickham cohort study showed a mean annual incidence of hypothyroidism of 35 cases per 10,000 surviving women and 6 cases per 10,000 surviving men, during a follow-up of 20 years (2). The overall prevalence of hypothyroidism in the Third National Health and Nutrition Examination Survey (NHANES III) cohort was 4.6% (3). In iodine-sufficient countries, the prevalence of hypothyroidism ranges from 1 to 2%, rising to 7% in subjects aged between 85 and 89 years (4). A 5-year study carried out in Australia highlighted a prevalence of subclinical hypothyroidism of 5.0% (5). Chronic lymphocytic thyroiditis (or Hashimoto's thyroiditis) represents the most common cause of primary hypothyroidism, accounting for around 50% of all cases. Other causes are iodine deficiency, radioiodine ablation, and surgery, that may be responsible for hypothyroidism in elderly hospitalized patients (6). Administration of amiodarone, antibacterial solutions or lithium can also be responsible for thyroid insufficiency (7).

Table 1. Major modifications in the aging thyroid.

Structural modifications	
	↑Size microfollicles
	↑Colloid cysts
	↑Lymphocytes infiltration
	↑Number of nodules
	↑Fibrosis
Hormonal modifications	
	Normal FT4 levels (↓secretion ↓degradation)
	Low-limit range FT3 levels
	↑rT3 levels
	↑TSH levels (<6.0 μUI/ml, 97.5th percentile over 70 years; <7.5 μUI/ml, 97.5th percentile over 80 years)
	↓bioactive TSH / immunoreactive TSH

Hypothyroidism may be classified as overt or subclinical (increased TSH with normal FT4 and FT3 levels). Subclinical hypothyroidism is common in elderly subjects and is associated with a number of clinical manifestations ranging from tiredness to cognitive impairment and coronary heart disease (8). Older patients require reduced dosages of levothyroxine to attain euthyroidism compared to younger patients, probably as a result of modifications in body composition or endocrine status occurring with age (9). The recent Institute for Evidence-Based Medicine in Old Age (IEMO) 80-plus thyroid trial aimed to investigate the effects of levothyroxine for 145 patients over 80 years with subclinical hypothyroidism (TSH ≥4.6 and ≤19.9 mU/L and FT4 within laboratory reference ranges). The results of this randomized clinical trial are expected to shed light on the multimodal effects of levothyroxine treatment in 80-plus subjects, highlighting benefits and potential adverse effects (10). The normal reference range of serum TSH in adult subjects is 0.4–4.5 mIU/L (11). In primary hypothyroidism it is possible to observe high TSH, low total T4, low FT4, high cholesterol (due to a reduction in the synthesis of LDL receptors), high creatine kinase (CK) levels due to skeletal muscle involvement and thyroid antibodies in case of Hashimoto's disease (12, 13). Secondary (or central) hypothyroidism (SH) is caused by a dysfunction of the pituitary gland or the hypothalamus, and is characterized by both decreased TSH secretion and low levels of thyroid hormones (Figure 1). SH can be classified into secondary and tertiary according to a pituitary or hypothalamic origin, respectively. Possible causes of SH include pituitary adenomas, and the subsequent surgical and/or radiotherapic treatment (14–16).

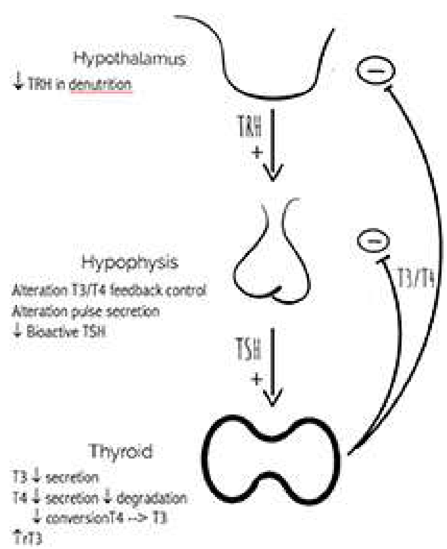

FIGURE 1. Age related changes in the hypothalamic-pituitary-thyroid axis.

Non-thyroidal illness (NTI), or low-T3 syndrome, is a condition that occurs during acute stress or critical illness, due to a block in the peripheral conversion of thyroxine. NTI is a well-recognized negative prognostic factor in patients with severe acute disease. A recent study showed an association between preoperative hypothyroidism and post-operative arrhythmias in older patients, thus suggesting the utility of preoperative T3 evaluation and preoperative supplementation (17, 18). Low T3 syndrome is very common in the hospitalized older population, emerging as an independent predictor of short-term survival, thus suggesting FT3 determination as mandatory in the workup of these patients (19). The aim of this review was to summarize the role of thyroid function as a predictor of surgical outcomes in the elderly.

MATERIALS AND METHODS

To retrieve the articles, an extensive literature search was performed using the databases of Medline through PubMed, Scopus, and Google Scholar from January 2000 to September 2018. The search terms were “elderly,” “older adults,” “hypothyroidism,” “thyroid surgery.” Particular emphasis was given to implications of hypothyroidism on the surgical risk in elderly subjects. Manual search was also performed on numerous textbooks of medicine, endocrinology, and critical care.

Clinical Features and Complications of Hypothyroidism in the Elderly

Thyroid-related symptoms are sometimes comparable to physiological manifestations of the aging process. In fact, signs, and symptoms of hypothyroidism are often less recognizable in elderly patients compared to younger subjects, thus posing diagnostic challenges (20). Nevertheless, hypothyroidism may be related with many symptoms which can be present in critical patients, such as cognitive impairment, cardiovascular, gastrointestinal, and hematological alterations, and eventually myxedema coma which is a severe and life-threatening condition in older adults. It is not possible to confirm a diagnosis of hypothyroidism based only on clinical symptoms, without TSH and FT4 assessment (21). In general, elderly subjects suffering from hypothyroidism may show classic symptoms, but complaints are often less specific than those described by younger hypothyroid patients (22). Doucet et al. compared the rate of 24 clinical symptoms of hypothyroidism between elderly patients and younger patients, and showed that fatigue and weakness were reported by more than 50% of the elderly patients, while increased sensitivity to cold, weight gain, paresthesiae, and muscle cramps were less common in the elderly (23). Carlè et al. compared the efficacy of hypothyroidism-associated symptoms in predicting overt hypothyroidism in different age groups, and observed that only dyspnea, fatigue and wheezing were more prevalent in elderly patients (24). Hearing loss, ataxia, and dysgeusia are neurological symptoms frequently described in hypothyroid older patients (25). Especially among elderly, neuropsychiatric symptoms such as memory loss or depression (26), dermatologic or rheumatologic disorders (27), are commonly described and it is difficult to related them to hypothyroidism. The list of signs in elderly with hypothyroidism may also comprise dry skin, hair loss, low heart rate, increased diastolic blood pression, pallor, and hoarseness (28). Cooper et al. observed that patients with subclinical hypothyroidism had a more elevated prevalence of symptoms as compared to controls with normal thyroid function (29). Another study by Kong et al. showed that the most common symptoms in women with subclinical hypothyroidism were fatigue (83%), weight gain (80%), and anxiety (50%) (30). Myxedema coma is a life-threatening condition due to hypothyroidism, which is characterized by a severe multiorgan failure (31). Myxedema coma is a rare disease, with an incidence of 0.22 per million per year in Europe (32). Most cases of myxedema coma occur in subjects 60 years and older (33) and are generally caused by precipitating factors that include exposure to cold, infections (i.e., pneumonia and urosepsis), withdrawal of thyroid supplements, and drugs (i.e., amiodarone or lithium) (34, 35). The diagnosis of myxedema coma is made on the combination of clinical manifestations and laboratory findings. The clinical presentation may include hypothermia, hypotension, bradycardia, congestive heart failure, hypoxaemia and hypercapnia, lethargy, and coma (36). Some patients show pericardial effusions, that are generally not hemodynamically significant. Laboratory assessment may show severe hypothyroidism, hypoglycemia, hyponatremia, and adrenal insufficiency (37). Myxedema coma represents an endocrine emergency with a mortality rate of nearly 40% (38). Major risk factors of mortality consist of older age, cardiovascular disease, and treatment with high-dose thyroid hormone (39).

Preoperative Screening and Treatment Considerations

The effects of thyroid dysfunction are various and may complicate surgical procedures and post-operative recovery. Currently, there is no recommendation for routine screening to detect thyroidal disease in patients with no previous history of thyroid dysfunction. A preoperative TSH assessment should be performed in subjects with suspected thyroid disease or with known hypothyroidism (or hyperthyrodism) to optimize treatment before surgery (40).

There is general consensus about the utility to post-pone elective surgery until adequate treatment with thyroid hormone has achieved euthyroidism. At the preoperative stage, LT4 should be administered in a titrated manner to normalize the thyroid function. The optimal preparation period before elective surgery should range from 2 to 4 weeks. Patients older than 60 years, especially with coronary disease, should not be given full dose of LT4 at the beginning (40). In such patients, the starting dose is generally 25 μg per day, which increases every 2–6 weeks until the achievement of euthyroidism. In patients unable to take LT4 orally for more than 5 days after surgery, intravenous levothyroxine should be given at a dose between 60 and 80% of the oral dose (41).

Implications of Hypothyroidism on the Surgical Risk

Preoperative recognition of hypothyroidism is crucial to reduce surgical and anesthesiological complications (41). Surgical trauma may influence the activity of the pituitary-thyroid axis, and thyroid hormones are secreted after surgery as a response to stress (42).

Anesthetic agents rather than surgical stress may be considered the main cause for the changes in plasma thyroid hormone concentrations during the intraoperative period (43). Many studies showed that adequate thyroid hormone levels are required to achieve optimal outcomes from any kind of surgical intervention (44). Correction of hypothyroidism, after replacement treatment, usually leads to the regression of pathophysiologic modifications due to low circulating thyroid hormone. Therefore, the achievement of euthyroidism represents the goal before elective surgery, in order to prevent the risk of complications. In non-elective surgery, a careful risk-benefit evaluation in hypothyroid patients before surgical treatment is needed. Only few randomized clinical trials investigated the association between NTI and adverse surgical outcomes so far (17) (Table 2).

Table 2. Main studies on the association between preoperative hypothyroidism and surgical outcomes.

References	Patients	Drug administration	Main results
Klemperer et al. (45)	142 patients undergoing CABG	Triiodothyronine $n = 71$ (Mean age 66 ± 10 years) or placebo $n = 71$ (Mean age 68 ± 9 years)	↑cardiac output ↓systemic vascular resistance No changes in post-operative mortality and morbidity
Worku et al. (18)	821 patients undergoing cardiac surgery Euthyroid $n = 682$ (Mean age 65.7 years) Hypothyroid $n = 77$ (Mean age 63.9 years)	None	Preoperative hypothyroidism was associated with post-operative atrial fibrillation
Cerillo et al. (46)	806 patients undergoing CABG Mean age 67.5 ± 9.6 years	None	Low T3 is a strong predictor of death and low cardiac output in CABG patients
Park et al. (47)	260 patients undergoing CABG Euthyroid $n = 224$ (Mean age 65.3 ± 9.4 years) SCH $n = 36$ (Mean age 65.4 ± 11.4 years)	None	↑post-operative atrial fibrillation
Jaimes et al. (48)	626 patients undergoing first-time isolated myocardial revascularization surgery Euthyroid $n = 313$ (Mean age 63 years) Hypothyroid $n = 313$ (Mean age 68 years)	None	Hypothyroidism is a risk factor for the onset of post-operative fibrillation

CABG, coronary artery bypass grafting; SCH, subclinical hypothyroidism.

A study by Park et al. did not show significant differences between patients with subclinical hypothyroidism and euthyroid patients undergoing a cardiovascular surgery procedure, as regards respiratory and cardiovascular complications, wound problems, leg infection, mediastinitis, and delirium. It was noteworthy that in the subclinical hypothyroidism group there was an increase in the rate of post-operative atrial fibrillation (47). Another study reported an association between preoperative hypothyroidism and post-operative atrial fibrillation in young-old patients, thus suggesting that preoperative hypothyroidism could be helpful for selecting those patients who would take advantage from preoperative replacement therapy in the prevention of post-operative atrial fibrillation (18). Furthermore, it has been observed a strong association between NTI at admission and increased risk of post-operative myocardial dysfunction and death in subjects undergoing coronary artery by-pass grafting (46).

A study by Weinberg et al. reported the effects of anesthesia and surgery in 59 hypothyroid patients compared with 50 euthyroid patients. The two groups did not show significant differences as regards duration of surgery or anesthesia, lowest temperature and blood pressure recorded during surgery, time to extubation, incidence of arrhythmias, need for vasopressors, fluid and electrolyte imbalances, sepsis, pulmonary and myocardial infarction, bleeding complications, or time to hospital discharge. After the analysis of subsets of thyroxine levels (thyroxine level <1.0 µg/dL, 1.0 to <3.0 µg/dL, and >3.0 µg/dL), the authors concluded that there was no evidence to post-pone surgery until the correction of mild or moderate hypothyroidism, whereas there was poor evidence to make a recommendation for patients with severe hypothyroidism (49).

Patients with hypothyroidism show slower drug metabolism and are exposed to the risk of an overdose of anesthetics and other medications used during the surgical treatment (50). The anesthesiological management of hypothyroid patients may face important clinical challenges, such as the presence of impaired baro-receptor reflex mechanism, depressed myocardial function, depressed ventilatory drive, and low glycaemia (51). There is no general consensus about surgery planning time for mild or moderate hypothyroidism as concerns anesthesia practice (52). However, in hypothyroid patients low-dose regional anesthesia could represent an option for minor surgery procedures (52). There is evidence that spinal, epidural or thiopental anesthesia could have low effects on thyroid hormones compared to general anesthesia; thus these methods should be taken into account in patients with thyroid function disorders, according to the type of surgical intervention needed.

CONCLUSIONS

It is recommended to post-pone elective surgery in elderly patients with hypothyroidism until an euthyroid state is achieved. If patients need urgent or emergent surgery, it is recommended to proceed with surgery only if they have mild or moderate hypothyroidism. Replacement therapy should be started preoperatively and there should be growing attention to the possible occurrence of minor post-operative complications in hypothyroid patients. As suggested by the American Thyroid Association (ATA), the treatment in elderly patients should be initiated at low doses with slow titration based on serum TSH evaluation. Elderly patients show higher normal serum TSH ranges; thus, higher serum TSH targets may be necessary as a patient ages. The suggested target serum TSH in people age 70–80 years is 4–6 mIU/L (8). Further clinical trials assessing surgical management in older hypothyroid patients are firmly required.

AUTHOR CONTRIBUTIONS

MV, AMB, AB and FB conceived the review. MV and AMB wrote the manuscript and realized the figures and tables. SDS, SL, CB, RC and ESDV performed the literature search and critically revised the manuscript for important intellectual content.

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REVIEW
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Osteoporosis and Sarcopenia Increase Frailty Syndrome in the Elderly

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Musculoskeletal aging is a major public health interesting and strain due to the significant demographic modifications in the population, and it is linked to high risk of falls, loss of autonomy in elderly individuals and institutionalization with small health outcomes. Thus, this pathological status is related to high morbidity and health care rates. Bone mass and muscle mass and strength increase during late adolescence and early adulthood but start to reduce noticeably from the fifth decade of life and are closely linked. Bone and muscle tissues were increasingly recognized, as endocrine target organs and endocrine organs themselves, interacting through paracrine and endocrine signals. During growth, bone mineral content closely correlates with muscle mass, and several evidences suggest that osteoporosis and sarcopenia present common pathophysiological factors and show the correlation between low bone mineral density and sarcopenia in both men and women. Then, sarcopenia and osteoporosis, typical features of aging, are often associated with each other and with the frailty syndrome. In particular, sarcopenia and osteoporosis are major contributors to disability and frailty and the common denominators are age-related chronic inflammation, changes in body composition and hormonal imbalance. Frailty syndrome is characterized by a reduced response to stress, triggering the decline of the physiological functioning of the various systems. Frailty syndrome, typical of the older people, is frequently associated with a reduction in the quality of life and mobility. Falls often are the basis of reduced mobility and ability to perform the common functions of daily life and the increase in the number of institutionalizations. Moreover, the reduction of muscle mass, associated with altered muscle composition, fat and fibrous infiltration and alterations in innervations, and the increase in fat mass, have a synergistic effect on the increase in cardiovascular risk. The aim of this review is to analyze the pathophysiological mechanisms underlying the frailty syndrome and its association with sarcopenia and osteoporosis, and investigate possible intervention measures.

Keywords: osteoporosis, sarcopenia, obesity, frailty syndrome, aging, gender, physical activity, diet

INTRODUCTION

Musculoskeletal aging is a major public health interest and is strain typical of the demographic changes in the population. It is associated with high risk of falls, loss of autonomy in elderly people and institutionalizations with small health outcomes. This condition is therefore correlated with high morbidity and health care rates (1, 2).

Indeed, world population is aging and, worldwide, individuals over 60 are estimated to increase from 841 million in 2013 to more than 2 billion by 2050, with a proportional gain from 11 to 22%. However, often the increase in life expectancy is not an increase in “healthy life” expectancy, and these additional years are loaded with scarce health and disability (3).

Musculoskeletal aging has many causes, including age-related changes in body composition, inflammation, and hormonal imbalance. Furthermore, sarcopenia and osteoporosis are linked and commonly associated with aging, often leading to a frailty syndrome.

Frailty is a physical condition, typically observed in elderly people, characterized by a gradual and growing loss in the function or reserves of multiple physiologic systems, which increased vulnerability and inability to maintain or recover homeostasis after a destabilizing occurrence, such as fever, infection, surgery, falls, and homeostasis changes due to pharmacological therapies (4, 5). Then, frailty can be considered a biologic condition characterized by low resistance and response to stressors, as a consequence of a general decline which includes multiple systems and organs. The clinical signs of frailty are: body weight loss, sarcopenia, osteoporosis, declined physical activity, reduced balance and gait speed, reduced cognitive function, and alterated state of nutrition. Thus, frailty determines a high risk for reduced activities of daily living, for cardiovascular diseases, cancers, falls, limited mobility, and increases risk of hospitalization and mortality (6).

Fried et al. proposed a “physical” phenotype of frailty, consisting of five features for identification of frailty syndrome: weakness (evaluated by grip strength), slowness (evaluated by gait speed), reduced attitude to the physical activity, reduced energy (self-reported), and involuntary body weight loss. The presence of one to two features points a pre-frail condition while three or more indicates a frailty syndrome (7). Since frailty, defined by these criteria, has been correlated with adverse health outcomes (7), other more simplified models of frail phenotype were later developed using data from the Study of Osteoporotic Fractures and the Three-City Study. Their predictive potencies for disability and mortality were either similar or improved compared to the original Fried model of frailty (8, 9).

On the other hand, a “multi-domain” of frailty phenotype exists, proposed by Rockwood et al. (10) who developed the Frailty Index (FI), calculated from an extensive questionnaire of diseases and ill-health. FI is based on identified deficits in several domains such as cognition, mood, motivation, communication, mobility, balance, activities of daily living, nutrition, social resources, and several other comorbidities. FI is reported as a ratio of prevalent deficits to the total number of potential deficits, and the greater the rate of deficit a subject has, the more likely this subject is to be frail. This index is considered to be highly predictive of high risk of mortality and institutionalization (10).

A reduction of muscle mass and strength with a corresponding increase of fat mass in the elderly might synergistically increase the risk of cardiovascular diseases (11). In fact, during aging, fat mass increases and its distribution changes with a decrease in subcutaneous fat and an increase of visceral fat, leading to a new nosographic entity named sarcopenic obesity (3).

The alterated fat distribution observed in aged people and/or in obese subjects is in fact characterized by intermuscular and intramuscular fat infiltration, both associated with a decline in muscle and mobility function and considered significant predictors of frailty and several comorbidities such as insulin resistance, diabetes, cardiovascular diseases, stroke, spinal cord injury, and chronic obstructive pulmonary disease (12). The mechanism(s) by which intermuscular and intramuscular fat negatively influences muscle function is actually unknown. However, the release of pro-inflammatory citokines from ectopic fat might provide this negative association (13), as well as the increased expression of Perilipin2 (Plin2), a protein associated with lipid droplet deposition, age and low muscle strength and thickness, both in humans and animal models (14) (Figure 1). In addition, as a consequence of fat muscle infiltration, motor units often undergo denervation and fast type II muscle fibers switch to slow type I fibers, leading to decreased muscle mass and strength (15, 16). A recent, interesting study shows that older patients who underwent extended high-intensity resistance training after hip fracture had improved quadriceps muscle mass and strength, while intramuscular fat remained unchanged (17).

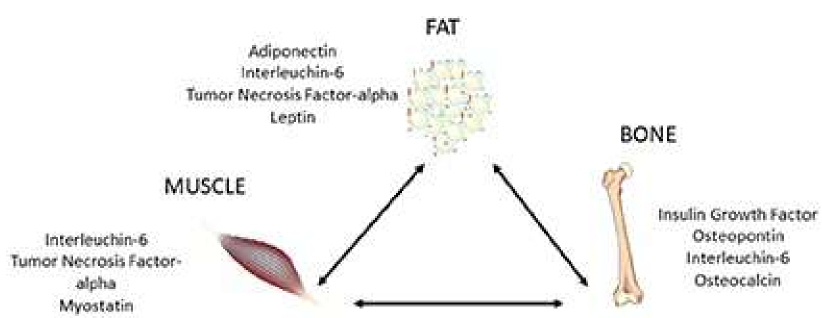

FIGURE 1. Physiopathological basis of frailty. Aging, reduced physical activity, nutrients intake, hormone imbalance, and chronic inflammation worsen pathological conditions as sarcopenia, obesity, and osteoporosis, which in turn lead to frailty syndrome.

Muscle mass and strength increase during late adolescence and early adulthood, and generally start to decrease from the fifth decade of life. In particular it decreases annually by 1–2% from the age of fifty, by 1.5% from the age of 50–60 and by 3% thereafter (3). Moreover, the decrease in muscle mass and strength negatively affect bone mass, which also declines during aging, causing osteopenia and osteoporosis. Aged, post-menopausal women have an increased risk of both osteoporosis and sarcopenia (1, 2), and present a loss of muscle performance more rapidly than men, suggesting a protective role of estrogens in the maintenance of muscle homeostasis beside their known role in skeletal health maintenance (18). In men, there is no androgen decline comparable to menopause, however, lower testosterone levels correlate with lower protein synthesis, loss of muscle mass, and sarcopenia (19).

Other important factors might affect muscle well-being are decreased protein intake and low-grade chronic inflammation, which often characterize aging. In elderly people, protein intake and protein synthesis are reduced and the production of pro-inflammatory factors is increased (18), and this low-grade inflammation further contributes to the anorexia of aging and correlates with reduced mobility and impaired cognitive function, representing an independent risk factor for disability (3).

Finally, a new interdisciplinary field named “geroscience” aims to understand the relationship between aging and chronic age-related diseases and geriatric syndromes. It is based on epidemiological evidence and experimental data that aging is the major risk factor for such pathologies, and assumes that aging, age-related diseases and geriatric syndromes share a common set of basic biological mechanisms. Geroscience assumes that an individual will follow an accelerated or de-accelerated aging process through his/her genetic background, interacting lifelong with environmental and lifestyle factors. It is clearly urgent to identify markers capable of distinguishing between biological and chronological age to identify subjects at higher risk of developing unhealthy aging. Recently, some authors have proposed the use of DNA methylation, N-glycans profiling and gut microbiota composition over the available disease-specific markers (20).

The aim of this review was to analyze reciprocal pathophysiologic relationships between the frailty syndrome, sarcopenia and osteoporosis. We performed Medline searches on the pathophysiology of the aforementioned geriatric syndromes, we describe common/joint disease mechanisms and present the concept of sarcopenic obesity. The final section of our review is dedicated to potential intervention measures.

SARCOPENIA AND OSTEOPOROSIS AS CONCEQUENCES OF AN ALTERED MUSCLE-BONE CROSS-TALK

Interestingly, data from many studies show that frailty is strictly associated with sarcopenia, osteopenia or osteoporosis, and falls (6), and these studies show that balanced physical activity and diet interventions are the focus of treatment, even though the treatment strategy depends on the specific frailty domain shown by the subject (3).

Several studies also show the correlation between low BMD and sarcopenia in both men and women (21). In the European Male Aging Study, in which 679 men aged 40–79 years were evaluated, sarcopenia was associated with osteopenia and osteoporosis (22), and similarly, high lean muscle mass and strength were positively associated with BMD. Whereas, sarcopenia was associated with low BMD and osteoporosis in a study of 17,891 subjects from various ethnicities (23). Moreover, a recent, interesting study by Locquet et al. showed, in a population of 232 elder people (age>75 years) of both sexes, that the decline in muscle performance was related to the decline in bone microarchitecture, and that subjects with incident sarcopenia had an approximately 5-fold increased risk of concomitantly developing osteoporosis, showing a dynamic relationship between impaired muscle and bone health, with an obvious association between the concomitant incidences of osteoporosis and sarcopenia (24). Finally, several studies showed that sarcopenia is an independent predictive factor of high fracture risk besides BMD and other clinical conditions (25), and that an association exists among sarcopenia, risk of falls and osteoporotic fractures (26–28). It is clear that the two conditions, sarcopenia and osteoporosis, are closely correlated, and that their combination leads to exacerbation of negative health effects and to frailty syndrome development (29).

Skeletal muscle is the body's scaffolding and allows movements and locomotion. Skeletal muscle can be affected by aging, low nutrition, disuse, inflammation, and hormone imbalance, that lead to loss of muscle mass and strength, a condition named “sarcopenia,” which is associated with frailty, cachexia, osteoporosis, metabolic alterations, and mortality. Like frailty, sarcopenia is strongly associated with loss of function and negatively influences people's ability for independent living, that might determine isolation and cognitive alterations, with an increase in the assistance care costs (3).

In 1989, Rosenberg first proposed the term “sarcopenia” (from the Greek “sarx” for flesh and “penia” for loss) to define the typical age-associated decrease in muscle mass (30), but during the last few decades its definition has been enlarged to include reduced muscle mass and reduced muscle function, and the consensus definition of sarcopenia is still under debate. Low lean mass, muscle strength and weakness are the main criteria considered to define sarcopenia proposed by the European Working Group on Sarcopenia in Older People (EWGSOP) and The Foundation for the National Institutes of Health (FNIH) Sarcopenia Project. Other proposed criteria include those from International Working Group (IWG), European Society for Clinical Nutrition and Metabolism Special Interest Group on cachexia-anorexia in chronic wasting diseases (ESPEN) and Society of Sarcopenia, Cachexia, and Wasting Disorders (SCWD) (31). Regardless of the definition, all scientific societies agree that the preservation of muscle strength and power with advancing age is of high clinical significance. However, despite the preponderance of scientific investigations that have continued to focus primarily on determinates of skeletal muscle size, recent longitudinal and intervention-based studies have clearly demonstrated that muscle atrophy is a relatively small contributor to the loss of muscle strength, and that exogenous supplementation of androgens or growth factors have yielded an increase in muscle mass but only marginally improved muscle performance. Then, on the basis of these observations, in 2008 Clark and Manini proposed the term “dynapenia” (*dyna* refers to “power, strength, or force” and *penia* refers to “poverty”) to define the age-related loss of muscle strength and power (32). Of course, dynapenia both in association with sarcopenia and as an independent factor, increases the risk of poor physical performance, disability and even death.

The pathophysiological basis of muscle mass and strength loss include a variety of factors and pathways, such as environmental factors, hormonal alterations, motor-neurons and muscle fibers loss, decreased protein synthesis and/or increased protein catabolism, activation of inflammatory pathways, reduction in satellite cell counts, and mitochondrial dysfunction and/or reduction (31).

During aging, muscle fibers decrease in size and number and alterations in skeletal muscle composition occur. As mentioned above, an increased fat infiltration in skeletal muscle is described, which significantly alters muscle quality and performance, and leads to a sarcopenic obesity (33–35). The prevalence of obesity in association with sarcopenia is increasing in adults over the age of 65 and older, leading to a high risk of synergistic complications from both sarcopenia and obesity (36). In sarcopenic obesity the excess of adipose tissue determines a dysregulated production of several adipokines which, in association with senescent cell- and immune cell-derived cytokines, create a local pro-inflammatory status. In addition, obese adipose tissue, through the excessive lipids production and their alterated storage, favors fat muscle infiltration and insulin resistance leading to pro-inflammatory myokines secretion, which in turn induces muscle dysfunction by auto/paracrine manner. Finally, these myokines, by endocrine manner, exacerbate adipose tissue inflammation and support chronic low-grade systemic inflammation, establishing a detrimental vicious circle triggering and maintaining sarcopenic obesity development (37). The increased body and muscle fat are associated with insulin resistance and low-grade chronic inflammation, with an increase in many specific and unspecific inflammatory parameters, like C reactive protein (CRP), fibrinogen, interleukin-6 (IL-6), and tumor-necrosis-factor alpha (TNF-α), which lead to the decrease in both muscle mass and strength, and to bone loss (35) (Figure 2). Moreover, an alteration in mesenchymal stem cell differentiation is observed, that is characterized by a high differentiation in adipocytes, which leads to a reduced muscular renewal (35) and Figure 3.

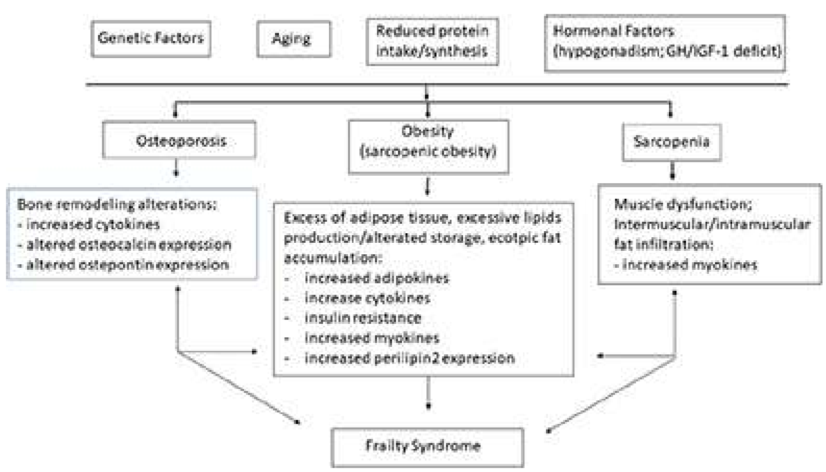

FIGURE 2. Schematic representation of molecular basis of the complex fat-bone-muscle cross-talk.

FIGURE 3. MicroCT image of the trabecular structure of a vertebral body of a C57Bl/6J mouse aged 12 month.

Also, sex steroids are involved in the pathogenesis of sarcopenia. In fact, the reduced estrogen levels after menopause amplify the increase in inflammatory markers (IL-6 and TNF-α), and since myocytes expressed estrogen-β receptors, a direct effect of estrogens on muscle mass has been proposed (38, 39). However, conflicting data exist about hormone replacement therapy (HRT) and its possible use for the prevention of the musculoskeletal aging (40). Finally, a decline in androgen levels may also play a role in the pathogenesis of sarcopenia, both in men and women (41).

Mitochondrial reactive oxygen species (mtROS) are tightly linked to oxidative stress in age-related muscle mass and strength. The deposition of mtROS in aged muscle determines tissue damage, muscle atrophy, muscle dysfunction, and increases in fibrous tissue (42). Moreover, mitochondria act directly on apoptosis, and their alterations and mtROS promotes cell degradation, the reduction of muscle fibers, and muscle atrophy (43). Also, an increased mitophagy has been associated with muscle atrophy (44).

Finally, myostatin, which is a well characterized myokine and a member of the transforming growth factor-β superfamily, negatively modulates muscle mass and growth and, interestingly, increased myostatin levels appear to be associated with aging (45). Indeed, several studies reported that myostatin levels were increased in frail elder women and were inversely correlated with muscle mass (46–48). However, further studies are needed to better understand the relationship between myostatin and aging.

During aging, bone remodeling is reduced, leading to a negative bone balance and increasing the incidence of age-associated bone alterations, such as osteopenia and osteoporosis. In particular, osteopenia is a clinical term used to describe a decrease in bone mineral density (BMD) below normal reference values, yet not low enough to meet the diagnostic criteria to be considered osteoporotic while osteoporosis is a skeletal metabolism alteration that causes a loss of bone mineral density and quality. This leads to bone fragility and high fracture risk, and osteoporotic fractures are associated with high morbidity and mortality (21).

The incidence of osteoporotic fractures increases with age, and actually, in women over 80 the incidence of hip fractures is 30%, while the incidence of vertebral fractures is more than 40% (21). Then, elderly patients with osteoporotic fractures should be considered as frail subjects, with low post-fracture outcomes that lead to functional decline, loss of quality of life, and increased mortality, for the next 10 years after the fracture (49). Moreover, osteoporosis is often associated with sarcopenia, with similar consequences, such as physical impairment, institutionalization, and depression, all conditions that increase morbidity and mortality (7).

During the last decade, bone and muscle were increasingly recognized as interacting tissues, not only because of their local proximity and their integrated function for locomotion, but were recognized also as endocrine target organs and endocrine organs themselves (50–52). In fact, the two tissues interact by paracrine and endocrine signals and modulate their development and function from intrauterine life to old age, and a linear relationship between BMD and muscle mass at various ages exists (52–54).

During growth, bone mineral content and femoral circumference closely correlate with muscle tissue, and several evidences suggest that osteoporosis and sarcopenia present common pathophysiological factors, including hormonal imbalance, increased inflammatory cytokine activity, release of tissue-specific molecules, nutritional changes, and physical impairment (53–56). The muscle–bone cross-talk is supported by preclinical and clinical data (Figure 3), showing the presence of many tissue-specific factors released by muscle that modulate bone, such as insulin-like growth factor-1 (IGF-1), fibroblast-growth factor-2, IL-6, IL-15, myostatin, osteoglycin, irisin, and osteoactivin (52). Interestingly, many factors such as myostatin, TNF-α, IL-6, and ROS that, as described above, are involved in the pathogenesis of sarcopenia are also regulators of bone remodeling, and thus are relevant for osteoporosis (56). However, actually there exists limited data about the modulation of bone on muscle, and both osteoblasts and osteocytes were shown to produce specific molecules, including prostaglandin E_2, osteocalcin, and IGF-1, which might impact skeletal muscle cells (52).

Finally, adipose tissue also interacts with bone and muscle, and obesity, sarcopenia, and osteoporosis could concomitantly exist. The increase in total and/or abdominal fat observed in obese subjects determines low chronic inflammation and hormonal imbalance which negatively affect both muscle and bone (50, 57). Indeed, people affected by sarcopenic obesity have a high risk of osteoporosis and fragility fracture, as well as other metabolic alterations resulting from changes in their body composition closely associated with high morbidity and mortality. These considerations of course emphasize the importance to strictly monitor bone health in sarcopenic obese subjects, mostly during aging (57).

DO SARCOPENIA AND OSTEOPOROSIS LEAD TO FRAILTY SYNDROME DEVELOPMENT?

Frailty is often discovered by maladaptive response to stressors, causing functional decline and other serious adverse health outcomes (4). During the last decade, a large amount of data suggest many causes for the pathogenesis of the frailty syndrome, such as chronic inflammation, musculoskeletal and endocrine system alterations, nutritional changes, and physical impairment, leading to a vicious cycle characterized by a progressive muscle and bone loss, as well as fat gain (28) (Figure 1).

In particular, chronic inflammation is a key factor that contributes to frailty, both directly and indirectly through other intermediate mechanisms (58). The relationship between frailty and inflammatory markers is well known, and many studies conducted in elderly people support the effect of chronic inflammation and immune activation on the frailty syndrome development (59–61). Inflammatory molecules directly contribute to frailty or indirectly through its detrimental effects on musculoskeletal metabolism and endocrine system (62).

Sarcopenia and osteoporosis are major contributors to disability and frailty. The age-related chronic inflammation, often indicated as “inflammaging,” leads to the decrease in both muscle mass and strength and bone loss, such as sex steroids and GH decline (35). In 2000, Franceschi et al. described the phenomenon of inflammaging as part of the spectrum of immunosenescence, leading to muscle and potentially bone loss (63). Inflammaging is believed to be a consequence of a cumulative lifetime exposure to antigenic load caused by both clinical and sub-clinical infections, as well as from exposure to non-infective antigens. The consequence is an inflammatory response, tissue damage and the production of ROS which result in the release of additional cytokines, determining a vicious cycle driving immune system remodeling and favoring a chronic pro-inflammatory state (63).

Sarcopenia and osteoporosis are also promoted by an increase in body fat and alteration in its distribution. With age, subcutaneous fat decreases despite the increase of visceral fat and fat infiltration of muscle fibers (3). The age-related increased body fat and muscle fat infiltration promote insulin resistance and inflammation that, through a vicious loop mechanism, determines muscle and skeletal metabolism alterations and dysregulation in mesenchymal stem cell differentiation leading to sarcopenia and osteoporosis (35). Obesity is also associated with sarcopenia, osteoporosis and frailty in both men and women as demonstrated by several studies, likely due to adipose tissue involvement in the complex bone–muscle interaction (50). In this view, obesity, sarcopenia, and osteoporosis could concomitantly exist, and the increase in total and/or abdominal fat and the excess fatty acids in the muscle fibers have also been shown to interfere with normal cellular signaling and favor inflammation and hormonal imbalance affecting both muscle and bone (50, 57). Further, sarcopenic obese subjects have a high risk of osteoporosis, fragility fractures and chronic metabolic disorders, resulting from the changes in their body composition (58).

Finally, sex steroids and IGF-1 are essential for bone and muscle metabolic regulation (62) and, indeed, the role of sex hormones in the pathogenesis of sarcopenia and osteoporosis has been well documented. The rapid drop in estrogens after menopause and the gradual decrease of androgens in older men result in decreased bone and muscle mass, with an increased risk of fragility fractures and sarcopenia in both genders but with a different timing; at the same time the low sex steroid levels determine an increase of the inflammatory markers which are linked to both sarcopenia and osteoporosis (38, 39). Circulating levels of dehydroepiandrosterone sulfate (DHEA-S) and IGF-1 are also significantly lower in frail older adults as compared to non-frail individuals, and many other hormones, such as cortisol and vitamin D, have also been associated with sarcopenia and frailty in the elderly, suggesting a potential impairment of the GH–IGF-1 somatotropic axis, the hypothalamic–pituitary–adrenal axis, and other hormones on the basis of frailty syndrome (62).

The altered cellular and molecular signals and functions described above, which lead to sarcopenia, osteoporosis, and inflammation, seem to be linked in a circle manner and probably represent the common denominator favoring frailty and unhealthy aging. However, further clinical and biological investigations are needed to better understand the complex multifactorial etiology of frailty.

INTERVENTION MEASURES

Nutritional intervention and physical activity are two pivotal measures for the prevention and treatment of sarcopenia, and they act in a synergistic manner.

Physical exercise in middle age seems to reduce the development of sarcopenia in older adults and it is also the primary measure for maintaining muscle mass and strength, and performance in elderly, as has been shown in the ROAD study, an observational study conducted on 1773 older adults, followed for 4 years (64). The aim of this study was to investigate the possible association of physical activity of daily living with the incidence of certified need of care in the national long-term care insurance system in elderly Japanese population-based cohorts, showing that physical dysfunction in daily living is a predictor of the occurrence of certified need of care (64). Moreover, a recent meta-analysis, including clinical trials on varied physical activity interventions for sarcopenia, showed a statistically significant association between physical activity and sarcopenia and documented its protective role against sarcopenia development, as well as heart diseases, diabetes, osteoporosis and pulmonary diseases (65). In fact, physical activity improves body composition by increasing muscle mass, reducing body fat, and improving muscle strength and endurance. In addition, physical activity can also modulate immune function and the cardiovascular system and, thus, it should be considered an essential measure of therapeutic strategies of age-related sarcopenia. Aerobic exercise improves mitochondria functions, aerobic capacity, metabolic regulation, and cardiovascular function. Also, aerobic exercise decreases the expression of catabolic genes and increases muscle protein synthesis (66–68). Resistance exercise prevents muscle wasting by stimulating muscle hypertrophy and increases muscle strength by regulating the protein metabolism balance (69). However, no single type of exercise but the combination of aerobic and resistance exercises should be preferred to prevent and treat the potential molecular mechanisms of age-related sarcopenia (70).

During aging, energy requirements decline, as do food and energy intake (71). Reduced food intake in elderly determines weight loss, with consequential decrease of muscle mass and strength and physical impairment (72). The importance of balanced nutrition in older adults has been recognized for a long time, but only recently have studies been designed to explore the effects of nutrition on muscle mass and physical performance (73). These studies suggest that diet has an important role in the prevention and management of sarcopenia and several kinds of interventions have been tested. The nutrients that have been most closely linked to the development of sarcopenia and frailty are protein, vitamin D, antioxidant nutrients (like carotenoids, selenium, and vitamins E and C), and long-chain polyunsaturated fatty acids (74). The few studies performed to date seem to indicate that there is a protective role of protein supplementation against frailty syndrome and it is tempting to suggest daily 30 g protein supplements help to prevent frailty. However, it is well established that excess protein can also be harmful; therefore, specific individual characteristics should be considered before prescribing these supplements. On the other hand, the relevance of other nutritional interventions, such as vitamin D, omega-3, and medium-chain triglycerides, is much more scarcely researched in the literature (75). Therefore, new clinical trials are necessary to carry out effective nutritional interventions to prevent frailty development.

Pharmacological therapies for the prevention and treatment of frailty are represented by drugs used to control both osteoporosis and sarcopenia. Anti-osteoporotic agents are used to increase bone mass and reduce fracture risk, such as bisphosphonates, denosumab, and teriparatide, associated with calcium and vitamin D supplementation. Advanced age is associated with increased signaling through extrinsic and intrinsic apoptotic pathways in skeletal myocytes, favoring the development of sarcopenia. Several preclinical studies suggest that myonuclear apoptosis might provide a selective biological target for the development of preventive and therapeutic interventions against sarcopenia. Many strategies, such as calorie restriction, exercise training, and drugs determine the down-regulation of myocyte apoptosis counteract the development or worsening of sarcopenia and muscle dysfunction (76). In particular, to prevent and treat sarcopenia, the appropriate pharmacological strategy might include myostatin inhibitors and type II activin receptor inhibitors; follistatin; testosterone or selective androgen receptor modulators (SARMs); angiotensin-converting-enzyme (ACE), inhibitors, and ghrelin mimetics (77). Other hormonal therapies, such as GH, IGF1, and estrogens, have also been experienced, but no evident beneficial effects have yet been demonstrated (78). The approach to increase muscle mass is the same for either young athletes or elderly individuals, however, in elder adults the need for a prolonged treatment makes it difficult to treat sarcopenia because of compliance and safety. Finally, since preclinical and clinical studies have demonstrated an increased number of senescent cells in the bone microenvironment during aging, with a consequential alteration in bone remodeling due to an increased secretion of inflammatory markers, a potential approach might either eliminate senescent cells or impair the production of their inflammatory factors, representing a novel therapeutic strategy to prevent multiple age-related diseases (79).

To treat and/or prevent frailty damages, early identification of people at risk of sarcopenia and osteoporosis is important. The European Working Group on Sarcopenia in Older People 2 (EWGSOP2) and the International Conference on Frailty and Sarcopenia Research (ICFSR) task force have recently produced a consensus and evidence-based clinical practice guidelines for its definition, screening, diagnosis and management (80, 81). Moreover, the ICSFR task force evaluated the evidence behind several topics (definition of sarcopenia, screening and diagnosis of sarcopenia, physical activity prescription, protein supplementation, vitamin D supplementation, anabolic hormone prescription, medication under development), considering the quality of evidence, the benefit-harm balance of treatment, patient preferences/values, and cost-effectiveness, and strongly recommend treatment of sarcopenia with prescribed resistance-based physical activity and conditionally recommended protein supplementation or a protein-rich diet (81).

CONCLUSIONS

World population is aging and the increase in life expectancy is often unhealthy. In particular, musculoskeletal aging, which leads to sarcopenia and osteoporosis, has several causes such as changes in body composition, inflammation, and hormonal imbalance. Sarcopenia, osteoporosis, and more frequently, sarcopenic obesity are commonly associated with aging and frequently closely linked each other, often leading to the development of a frailty syndrome. Frailty syndrome favors an increased risk of loss function in daily activities, for cardiovascular diseases, cancers, falls, and mortality. As the number of elderly people continues to increase, it is important to identify people at risk of frailty early and to treat and/or prevent its damages, developing interventions that can promote a "successful aging." The complexity and heterogeneity of frailty syndrome requires a multidimensional clinical approach based on healthy nutrition, psychosocial well-being, regular physical exercise, and pharmacological measures, which seem to prevent and control chronic diseases affecting both life expectancy and quality of life, thereby reducing mortality. Of course, new basic and clinical studies are necessary to better understand the complex pathophysiological mechanisms leading to frailty and to carry out effective measures of interventions to prevent its development and treat its damages.

AUTHOR CONTRIBUTIONS

EAG, PP, and SM equally contributed to the preparation of the manuscript.

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REVIEW
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Role of c-Kit in Myocardial Regeneration and Aging

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c-Kit, a type III receptor tyrosine kinase (RTK), is involved in multiple intracellular signaling whereby it is mainly considered a stem cell factor receptor, which participates in vital functions of the mammalian body, including the human. Furthermore, c-kit is a necessary yet not sufficient marker to detect and isolate several types of tissue-specific adult stem cells. Accordingly, c-kit was initially used as a marker to identify and enrich for adult cardiac stem/progenitor cells (CSCs) that were proven to be clonogenic, self-renewing and multipotent, being able to differentiate into cardiomyocytes, endothelial cells and smooth muscle cells *in vitro* as well as *in vivo* after myocardial injury. Afterwards it was demonstrated that c-kit expression labels a heterogenous cardiac cell population, which is mainly composed by endothelial cells while only a very small fraction represents CSCs. Furthermore, c-kit as a signaling molecule is expressed at different levels in this heterogenous c-kit labeled cardiac cell pool, whereby c-kit low expressers are enriched for CSCs while c-kit high expressers are endothelial and mast cells. This heterogeneity in cell composition and expression levels has been neglected in recent genetic fate map studies focusing on c-kit, which have claimed that c-kit identifies cells with robust endothelial differentiation potential but with minimal if not negligible myogenic commitment potential. However, modification of c-kit gene for Cre Recombinase expression in these Cre/Lox genetic fate map mouse models produced a detrimental c-kit haploinsufficiency that prevents efficient labeling of true CSCs on one hand while affecting the regenerative potential of these cells on the other. Interestingly, c-kit haploinsufficiency in c-kit-deficient mice causes a worsening myocardial repair after injury and accelerates cardiac aging. Therefore, these studies have further demonstrated that adult c-kit-labeled CSCs are robustly myogenic and that the adult myocardium relies on c-kit expression to regenerate after injury and to counteract aging effects on cardiac structure and function.

Keywords: c-kit, cardiac stem cells, cardiac aging, cardiac regeneration, cardiac remodeling

INTRODUCTION

In developed countries, modern and up-to-date guidelines-recommended treatments based on solid clinical and basic cardiovascular research have significantly reduced the mortality for acute cardiovascular (CV) syndromes (1, 2). However, the improvement in primary treatment of cardiovascular syndromes bargained a steep surge of patients with chronic heart failure (CHF), a syndrome that nowadays has numbers similar to an epidemy and takes the highest toll on human lives among CV diseases (3).

Indeed, during acute life-saving interventions, most patients irreversibly develop myocardial injury, from which CHF develops. CHF has no available curative therapies and the prognosis for patients is poorer than that for most cancers, having an average survival of only 3–5 years after its onset (1–3). There are almost 40 million patients worldwide with HF that account for a significant part of the annual hospital admissions and that absorb several billions of dollars to the USA healthcare. Similar number of patients and annual costs are emerging to be found in the EU healthcare systems after several statistical analysis (1, 2). It follows that HF treatments currently in use are only symptomatic if not just palliative when considering mortality as main endpoint—with heart transplant as only valid yet practically un-available solution to overcome it. It is imperative indeed to develop technologies to better understand and to monitor CV diseases, their symptoms and complications, with the aim to preserve/enhance the function of the surviving cardiomyocytes, while also to replace the lost cardiomyocytes, primary causes of CHF (1, 2).

Myocardial infarction, and ischemic heart disease in general, is the primary etiology of CHF (1, 3). Also in the cases of the structural cardiomyopathies, where the CHF is of non-ischemic origin, the primary issue is the lack of the myocardium to undergo a robust cardiomyocyte replacement (2). On surprisingly, therefore, regenerative biology/medicine has raised with the goal to find an effective and broadly available therapy to refresh the contractile muscle cells lost and/or permanently dysfunctional in consequence of the primary injury (2, 4). Unfortunately, the predominant skepticism about the intrinsic endogenous regenerative capacity of the adult mammalian heart, including the human have produced often contradictory approaches to perform myocardial repair/regeneration (2).

Until sufficient scientific data are obtained to eventually overcome this widespread skepticism, whereby hard clean and clear data remove the need for interpretations and opinions, no clinical repair or regeneration protocol will be ever able to answer the question of whether it is feasible to functionally regenerate the failing human heart (2).

BIOLOGY OF THE ADULT HEART: THE OLD PARADIGM

The adult cardiomyocytes (CMs), terminally differentiated cardiac parenchymal cells, permanently withdrawn from the cell cycle with no capacity to replicate, have been classically defined as *elementi perenni*, similarly to neurons, and thus believed to last a lifetime (2, 5, 6). The main underlying and ensuing biologic dogma was and still practically remains that, when the heart is subjected to a prolonged work overload or to a diffuse and/or segmental injury, the CMs respond increasing their size, becoming hypertrophic to accommodate a larger number of its sarcomeres to sustain the increased work or just die (2).

This static view of the biology of the adult hearts postulates that from cradle to grave no new CMs are therefore added and it turns that to maintain an equilibrium for the heart to properly function and sustain the systemic circulation throughout life, CM death is a rare, if not negligible, event (2). Thus, under this dogmatic view, post-natal life of the heart is not ruled by a cell homeostasis process where cardiac muscle cells die and are consequently replaced in response to wear and tear and/or injury (2).

On this basis, one of the first attempt, still ongoing, to obtain cardiac muscle regeneration has been and continues to be the re-activation of mitotic division of mature terminally differentiated CMs (7). However, genetic modification of the myogenic differentiation network and muscle cell identity of adult CMs to force their division to produce a robust number of new CMs has mainly resulted in increased polyploidy and/or death, both *in vitro* and *in vivo* (2, 7–9). On the other hand, experimental approaches conducted in order to increase CM division, which have been proven to foster beneficial functional *in vivo* effects (9, 10), are not necessary to clearly rule out whether the detected new cardiomyocyte formation is the product of the division of pre-existing terminally differentiated CM or of myocyte progenitors before their terminal differentiation (2). Moreover, the heart is the organ of the adult human body less affected by neoplastic transformation (11), which has been classically referred to the "stubborn" terminally differentiated state of the adult CMs. It logically turns that the inhibition and/or removal of the CM inhibitory cell cycle checkpoints maintaining their differentiated state in the adult heart in the myocardium will run the high risk of breaking the intrinsic protection of the adult heart from neoplastic development (2).

Overall, the classic dogma of the biology of the adult heart considered nil the regenerative potential of the adult myocardium and its response to increased workload limited to CM hypertrophy. Under these biologic tenants, no effective protocol for myocardial regeneration could be developed unless exogenous effective regenerative agents were discovered and applied. Cardiovascular therapeutic research has been developed under this biologic umbrella up to today (2).

BIOLOGY OF THE ADULT HEART: THE NEW PARADIGM

The historic paradigm of mammalian CM terminal differentiation and permanent withdraw from the cell cycle (2, 5–7, 12) started to be challenged by the evidence arising from few reports of sporadic new CM formation in the normal and pathological adult heart (2, 13, 14). As the number of this new CM formation was very small, and it had no biological basis to be mechanistically interpreted, they were disregarded as a curiosity or just an experimental artifact with no physiological significance (2).

The initial yet largely ignored detection of new CM formation in the adult mammalian heart has been recently confirmed and undoubtedly proven by cutting-edge molecular and genetic tracking techniques that have nowadays established that new CMs are continuously born in the post-neonatal mammalian heart, including the human (2, 15–20). However, despite this evidence, the quantification of this CM renewal in the adult heart remains highly debated and it is still widely regarded as a neglegible and therefore physiological useless phenomenon (2, 20). In adult healthy humans, using radioactive isotope decay, an annual CM turnover rate of ~0,5% has been reported through mathematical extrapolation (16, 21). In small mammals, the estimated range of CM annual turnover spans from 0.001 to 4%. Nevertheless, the reliability of all these estimates remain questionable simply because they are extrapolations and not diresct experimental measurements (2).

Nevertheless, while there is a lack of agreement about CM turnover rates, and myocardial regenerative response in general, there is a consensus that the heart response to damage is not sufficient to counteract the CM loss and dysfunction after myocardial infarction (MI) and in CHF (2). Because replacement of lost and injured CMs will continue to call for effective regenerative protocols, it is mandatory for the cardiovascular research community to define an experimental protocol that can directly and accurately quantify CM turnover in health and disease. Nonetheless, the undisputed existence of an intrinsic regenerative response with new CM formation in the adult myocardium is a solid basis to continue the search for its precise nature with the logical expectation that mastering its underlying mechanisms will provide new solutions to develop clinically meaningful protocols of myocardial protection, repair and/or regeneration (1, 2).

ADULT C-KIT^{POS} CARDIAC STEM CELLS: RETRACING THE STAGES OF THEIR DISCOVERY

A main obstacle hampering progress toward the development of effective cardiac regeneration protocols remains the lack of consensus about the origin and number of CMs which are born after the early post-natal period [(5, 6) days in the mouse, ~1 year in the human], when heart growth by CM replication stops and all CMs become terminally differentiated.

Since at least 2003, we have known that the mammalian heart, including the human, contains a pool of resident tissue-specific cardiac stem/progenitor cells, the endogenous CSCs (hereafter eCSCs when in the myocardium and CSCs when isolated and studied *in vitro*) (20, 22). Originally, the eCSCs have been identified as a small cardiac cell population through the expression of specific membrane markers, in particular the stem cell factor (SCF) receptor kinase c-kit (23), Sca-1 (24), and MDR-1 (25). *In vitro* and *in vivo* experimental tests have clearly shown that CSCs have all the characteristics expected from a tissue-specific stem cell: they are clonogenic, self-renewing and multipotent. They are indeed able to differentiate *in vivo* and *in vitro* into the main myocardial cell types –cardiomyocytes, endothelial and vascular smooth muscle cells and connective tissue cells (20).

Nevertheless, since the first report and despite a burgeoning and reproducible evidence characterizing tissue specific CSCs, several reports have questioned their existence (26–28). Unfortunately, oversimplification of the available data created the confusion whereby a single marker, i.e., c-kit and then Sca-1 in mice, became more important than the complex and exhaustive experimental approach used in the first place to prove that the heart harbors *bona fide* adult cardiac progenitor cells. Indeed, the experimental evidence that the adult heart contains a pool of cells that are clonogneic, self-renewing and multipotent was swiftly reduced, without an inch of supporting data, to a common notion that cardiac c-kit (or Sca-1) cells are the CSCs. On this basis, it was reported that c-kit^{pos} cells are robustly cardiogenic in the neonatal period while adult c-kit^{pos} cardiac cells are marginally, if at all, myogenic (29). It was also correctly shown that c-kit^{pos} cardiac cells are endothelial cells or mast cells, but this data, unsurprising because known since decades, was used to claim that c-kit^{pos} CSCs were just mast cells in the adult human heart (26–28). Despite the latter negative reports, after the first identification of eCSCs in the adult rodent heart (22), different groups have independently proven the existence of cells with similar characteristics and regenerative potential in practically all the mammalian species, including the human (30–37). Interestingly, the first report of the existence of cardiac tissue specific progenitors from human tissue was obtained by Messina et al. (32). These authors reported the isolation of undifferentiated cells that grow as self-adherent clusters (termed “cardiospheres”) from subcultures of post-natal cardiac human biopsy specimens and also from murine hearts. These cells are clonogenic, having the properties of adult cardiac stem cells. Indeed, they are capable of long-term self-renewal and can differentiate *in vitro* and after transplantation in SCID beige mouse in cardiomyocytes and vascular cells (32). Cardiospheres appear to have a bone marrow origin (38). c-kit^{pos} human CSCs (hCSCs) have been then isolated by explant culture technique and enzymatic digestion from myocardial samples of the four cardiac chambers of patients with ischemic and non-ischemic cardiomyopathy (39). These c-kit^{pos} hCSCs are self-renewing and clonogenic, and their capacity to generate clones from a single cell appears to be similar to their rodent counterparts. All the hCSCs clones tested express sizable levels of c-kit and they are negative for both hematopoietic and endothelial markers. When grown in suspension, these cells are able to form cardiospheres and, under adequate stimuli, they differentiate *in vitro* into cardiomyocytes, vascular smooth muscle and endothelial cells. Also hCSCs support myocardial regeneration when injected in immunodeficient rats with myocardial infarction. Further data on c-kit^{pos} cardiac cells have been obtained through c-kit^{BAC}-EGFP transgenic mice, in which EGFP expression is placed under control of the c-kit locus (40, 41). These reports showed that the myocardial c-kit-EGFP^{pos} cells increases in early post-natal growth, but declines in the first weeks after birth (40, 41).

c-kit-EGFP^{pos} cells isolated from neonatal hearts commit to all three cardiac lineages and, after plating in appropriate cardiac differentiation media, many c-kit-EGFP^{pos} cells differentiate into spontaneously contracting cells (40). When adult c-kit^{BAC}-EGFP^{pos} mice underwent coronary ligation to produce myocardial infarction, it was shown that c-kit expression increased significantly at 7 days after injury and declined by 4 weeks to baseline levels. Modest c-kit-EGFP^{pos} expression was observed in striated mature cardiomyocytes in the border zone (40). On the contrary, using a different approach, Fransioli et al. show elevated c-kit expression in the infarcted and border regions throughout 10 days after injury (41). Remarkably, they found c-kit-EGFP^{pos} cell recruitment to the area of injury, with their differentiation into cardiomyocytes, smooth muscle and endothelium (41).

In 2011 we showed that the adult pig myocardium, a frequently used and widely accepted pre-clinical large animal model for cardiac disease, harbors among the c-kit-labeled cells a significant fraction of blood-committed CD45^{pos} cells. On the contrary, c-kit^{pos}/CD45^{neg} pig cardiac cells behave as cardiac tissue specific stem/progenitor cells. These c-kit^{pos}/CD45^{neg} CSCs can be activated to proliferate when exposed to *in vitro* treatment with insulin-like growth factor (IGF)-1 and hepatocyte growth factor (HGF), while in differentiation conditions, they commit to cardiomyogenic lineage when treated with a combination of IGF-1 and HGF (42). These *in vitro* data was the basis to pre-clinical test of the intracoronary injection of small amounts of IGF-1 and HGF (a single dose ranging from 0.5 to 2 μg HGF and 2 to 8 μg IGF-1) to pigs subjected to acute myocardial infarction (AMI) by transient coronary occlusion. This experimental approach produces a robust activation of the eCSCs pool with ensuing robust cardiac muscle regeneration and cardiac function improvement (42).

The intracoronary IGF-1+HGF cocktail, in a dose-dependent manner, boosted myocardial regeneration but also it improved cardiomyocyte survival, and reduced both fibrosis and cardiomyocyte reactive hypertrophy. A single administration of IGF-1/HGF was sufficient to have a pronounced and durable beneficial effect, because through a paracrine effect on the endogenous myocardium activated a feedback loop on the targeted cells for the their production of cardiopoietic growth and survival factors. The histological changes correlated with a reduced infarct size and a better ventricular segmental contractility and ejection fraction when compared to control animals as assessed by cMRI (42). Similar positive effects were obtained when the IGF-1/HGF combination was administered trans-endocardially in pigs with a chronic MI using the NOGA system (43). Despite its effectiveness, the administration of IGF-1/HGF is insufficient for complete cellular maturation of the newly-formed CMs. Despite the beneficial effect of the therapy in reducing the scar area, pathological remodeling, and partial recovery of ventricular function, the growth factor combination must be still refined to include an improved cocktail that can generate a more rapid recovery of the ventricular mass capable to sustain a proper adult myocardium force.

Subsequently, to directly assess the endogenous regenerative potential of eCSCs we made use of a severe diffuse myocardial damage in the presence of a patent coronary circulation produced by high doses of Isoproterenol (ISO) that, unlike the segmental myocardial loss produced by permanent coronary ligation, spares the eCSCs (44). CSCs in culture are resistant even to the highest doses of ISO, which at the contrary kills the majority of primary cardiomyocytes at significantly lower doses (44). This resistance of CSCs to ISO is equally evident in animals *in vivo* so that eCSCs are available to respond to CM loss by the ISO-induced myocardial injury showing their regenerative endogenous potential. Using this experimental setting we have provided for the first time the evidence that eCSCs spontaneously and completely replace all the CMs lost following diffuse extensive myocardial damage which has killed ~10% of the ventricular myocytes. If the eCSCs are ablated, through the administration of the antimitotic agent 5-flurouracil (5-FU), there is absence of CM replacement and the lost contractile mass triggers terminal HF. If failing hearts devoid of their eCSC pool are treated with adoptive transfer of exogenous CSCs, progeny of a single syngeneic CSC, the endogenous CSC deficiency is corrected, and CM deficit is filled up with new CMs derived by the differentiation of the transplanted CSCs. This CSC-dependent regenerative process returns the myocardium to the *status quo ante*, the tissue damage is repaired reverting HF and normal cardiac function is restored. Subsequent selective ablation of these transplanted and engrafted CSCs and their differentiated progeny obtained by genetic activated suicide, rapidly sets the heart back in overt HF followed by death unless a new batch of CSCs is transplanted (45). Thus, using a variety of well-accepted genetic, cellular and molecular approaches we have provided the first evidence that the c-kit^{pos} eCSCs are necessary and sufficient for myocardial cell regeneration (45). Clearly this evidence is proof of concept because it does not question that this regenerative potential of the heart is inadequate to counteract the segmental loss by myocardial infarction.

Of note, to track c-kit^{pos} CSC fate *in vivo*, we generated a c-kit/Cre construct containing a short 5′ flanking region (~0.6 kb) of the c-kit promoter including the transcription initiation site (TIS) and the HS1 and HS2 sequences, important for cell specific expression (46) and Cre recombinase cassette inserted in frame with the ATG site within the exon 1 of c-kit. We demonstrated that this transgenic construct when released intra-myocardially through a lentiviral vector is not re-expressed either prior or after ISO injury in adult CMs (45). Moreover, when a similar c-kit/EGFP construct was injected into wild type mice, it correctly labeled c-kit interstitial cardiac cells including c-kit^{pos} CSCs but no mature CMs either in normal hearts or after ISO injury (45). Thus, this set of experiments were able to faithfully track the c-kit^{pos}CSC fate *in vivo* after ISO establishing their robust cardiomyogenic potential.

PHENOTYPIC IDENTITY OF TRUE ENDOGENOUS ADULT CARDIAC STEM CELLS: C-KIT EXPRESSION IS NECESSARY BUT NOT SUFFICIENT FOR THEIR IDENTIFICATION

The wrong notion that a single marker on its own, as c-kit, can identify a population of CSCs (47–51) has been the basis for apparently negative data about the nature and regenerative capacity of the “c-kit^{pos} cardiac cells” (47–51), which has created a significant and widespread skepticism on the validity of genuine data. It is worth noting here that the identification of a cell population expressing c-kit or whatever other single marker is clearly insufficient to score such cell pool as an homogenous c-kit positive stem cell population (52, 53). The adult heart contains a heterogeneous mixture of c-kit^{pos} cardiac cells. These c-kit-expressing cardiac cells are mainly mast and endothelial cells (52). Specifically, a myocyte-depleted preparation of pure c-kit^{pos} cardiac cells contains more than 90% of cells expressing blood and endothelial lineage markers, such as CD45 and CD31 (Lin^{pos}). These lineage-committed c-kit-labeled cells are not myogenic progenitors and do not possess stem cell properties *in vitro* and *in vivo* (52, 54). On the contrary, 10% of the total c-kit-expressing cardiac cells are negative for all the lineage-committed markers, including CD45 and CD31 among others. This c-kit^{pos}CD45/CD31^{neg} (also referred as lineage negative) cardiac cell population is enriched with a yet incompletely phenotypic-defined cell population that shows the prototypical stem cell properties *in vitro*: i.e., multipotent, self-renewal and clonogenesis (52, 54). Thus, to identify and isolate true multipotent CSCs is essential to eliminate, from the total c-kit-expressing cardiac cells, the most abundant lineage-committed (Lin^{pos}) cells. A negative sorting with CD45/CD31 antibodies followed by a c-kit antibody positive selection allows to obtain a cardiac cell population that is negative for CD45, CD31, and CD34 (Lin^{neg}) (52–54) and positive yet in different percentages for Sca-1, Abcg2, PDGFR-α, Flk-1, MDR-1, and CD166, all markers previously used by different groups to isolate endogenous resident cardiac cells with progenitor potential. Ten percent of the CD45^{neg}CD31^{neg}c-kit^{pos} cardiac cells population are clonogenic and are able to differentiate, *in vitro* and *in vivo*, into mature and functional CMs as well as vascular cells (52–57). A small fraction of freshly isolated CD45^{neg}CD31^{neg}c-kit^{pos} cardiac cells express pluripotency genes (Oct-4, Nanog, Klf-4, and Sox-2), but also stemness regulatory genes and typical transcription factors of the early stages of cardiac myogenic differentiation (Tert, Bmi-1, Gata-4, Mef2c, and Nkx2.5) (58–62). On the other hand, the lineage positive (CD45^{pos} and CD31^{pos}), Lin^{pos}c-kit^{pos} cardiac cells are negative for all these mutipotency genes and myogenic transcription factors and are solely able to differentiate into endothelial cells (54).

To narrow down the phenotype of true multipotent CSCs we obtained several clones from deposition of single CD45^{neg}CD31^{neg}c-kit^{pos} cardiac cells.

These cloned and sub-cloned Lin^{neg}c-kit^{pos} CSCs homogenously maintain a stable phenotype without signs of growth arrest, senescence or down regulation of stemness and cardiac gene expression. When grow in suspension, cloned Lin^{neg}c-kit^{pos} CSCs generate cardiospheres, and when were placed in established differentiation media for cardiomyocyte, smooth muscle and endothelial cell lineages, they differentiate into CMs, smooth muscle and endothelial cell lineages, respectively, at a significantly higher rate compared with the freshly isolated CD45^{neg}CD31^{neg}c-kit^{pos} cardiac cells (52, 54). The Lin^{neg}c-kit^{pos} cloned CSCs uniformly express c-kit, PDGF-Rα, CD166, SSEA-1, Nestin, Bmi-1, Tert, Gata-4, and Nkx2.5 and are negative for CD34, CD45, and CD31. All cloned CSCs also uniformly express the pluripotency genes Oct3/4, Nanog, Klf-4, and Sox-2 (22, 52–54, 63, 64). Thus, these experiments on single CD45^{neg}CD31^{neg}c-kit^{pos} cardiac cell-derived CSC clones prospect the phenotypic identity of the true endogenous CSC.

Cloned Lin^{neg}c-kit^{pos} CSCs respond *in vitro* to known cardiac morphogens like the Wnt/β-catenin and TGF-β/SMADs signaling pathways. Indeed, the canonical Wnt pathway, together with FGF and Hedgehog pathway, regulate cardiac progenitor cell proliferation in the mesoderm during embryonic life. On the contrary, Notch and non-canonical Wnt signaling regulate the differentiation processes during heart development (65–68). Interestingly, the cardiomyocyte differentiation program, in c-kit^{pos} CSCs, follows a step by step finely-regulated molecular cascade that is closely reminiscent of the known molecular program at the basis of the cardiac development from primary heart tube to the fetal/neonatal heart (54). *In vitro* administration of these specific cardiac morphogens allows to regulate the self-renewal potential and cardiomyogenic specification of CSCs to generate fully differentiated contracting CMs (52, 54, 69–72). CSCs express, Frizzled, the cell-surface receptor of Wnt/β-catenin canonical pathway, as well as its co-receptor, lrp-6, the low density lipoprotein receptor-related protein 6. Wnt-3a, Wnt-3a-conditioned medium, and bromoindirubin-3′-oxime (BIO) stimulate CSC expansion and clonogenicity, while canonical Wnt inhibition decreases CSC proliferation and clonogenicity *in vitro*. In contrast, Dickkopf-1 (Dkk-1) increases CSC myocyte specification, even though its effect is not sufficient to produce a fully differentiated contracting phenotype in culture. Additionally, clonogenic CSCs express TGF-β-R1, the cell surface receptor for TGF-β/SMAD signaling. In CM differentiation medium, BMP-2, BMP-4, TGF-β1, and Activin-A, factors that exert crucial roles in heart formation and CM specification during embryonic life, drive the expression of myogenic lineage markers in CSC culture increasing the number of cTnI^{pos} myocyte-committed cells (54, 73). Thus, CSCs respond to known cardiac morphogens. Inhibition of the Wnt canonical pathway and TGF-β family activation, each independently, promote cardiomyogenic commitment. Nevertheless, individual modulation of each of these cardiopoietic growth factors c(GFs) is insufficient to generate fully differentiated contracting CMs (74, 75). Remarkably, TGF-β family activation followed by the inhibition of the Wnt canonical pathway in a stepwise differentiation protocol induce full myogenic specification of CSC cultures with the appearance of spontaneously contracting cell clusters *in vitro* (52). Transcriptome comparison of RNA-seq data from CSCs, CSCs-derived CMs *in vitro*, neonatal CMs and adult CMs showed the highest similarity between CSCs-derived CMs and neonatal CMs. Therefore, the *in vitro* myogenic specification of clonogenic adult CSCs produces *bona fide* cardiomyocytes whose structural, molecular and functional maturity is nearly indistinguishable from neonatal mammalian cardiomyocytes (52, 54).

The regenerative capacity of adult endogenous $CD45^{neg}c\text{-}kit^{pos}$ CSCs has been evaluated using different rodent models of cardiac adaptations to stress and injury including diffuse myocardial damage inducing acute transient heart failure as well as physiological heart growth by exercise training (44, 76). After transplantation of *ex vivo* cloned and expanded $c\text{-}kit^{pos}$ cardiac cells from old heart rodent donors, it was originally demonstrated that the infarcted myocardium showed the appearance of islands of regenerated cardiac muscle tissue, composed of new cardiomyocytes and microvasculature (22). Recently, this evidence has been independently reproduced showing that administering a cell progeny derived from a single $CD45^{neg}c\text{-}kit^{pos}$ clonogenic CSC genetically marked with GFP in syngeneic rats after experimental AMI, these cells provide robust histological and functional myocardial regeneration. At 28 days after AMI, $CD45^{neg}c\text{-}kit^{pos}$ clonogenic CSC GFP^{pos} revealed high engraftment rate in the border/infarct zone, yielding myocardial regeneration with formation of new cardiomyocytes, capillaries and arterioles. Furthermore, this regenerative effect was associated with reduced pre-existing CM apoptosis and hypertrophy, significantly decreased scar size and left ventricle dilation. All together these regenerative and cardioprotective effects improved cardiac function (52, 54). On the contrary, the administration of total $c\text{-}kit^{pos}$ cardiac cells, which is mainly composed of $CD31^{pos}c\text{-}kit^{pos}$ endothelial committed cardiac cells, after AMI showed no regenerative nor cardioprotective effect on cardiac tissue histology and function with the detection of rare new cardiomyocytes. Most of the injected $c\text{-}kit^{pos}$ total cardiac cells acquired endothelial lineage specification (52).

Overall, these data show that only ~1% of the total myocardial $c\text{-}kit^{pos}$

population are real multipotent CSCs. The latter implies that c-kit is necessary but not sufficient to identify true adult CSCs. In order to assess the participation of CSCs in heart homeostasis/repair is therefore mandatory to identify this very small c-kit expressing regenerative population among the total c-kit^{pos} cardiac cells (54).

C-KIT FUNCTION IN ENDOGENOUS CSC BIOLOGY AND CARDIAC REGENERATION AND AGING

While no single marker, including c-kit, exclusively identifies a cardiac stem cell, there is agreement that expression of c-kit, a type III tyrosine kinase receptor, marks a developmental stage between cardiac mesoderm formation and differentiation into the specific cardiovascular lineages (60, 77–79).

c-kit receptor's function depends from its phosphorylation that is started by the binding of the stem cell factor (SCF), which is expressed as a soluble or membrane bound splice variant. C-kit activation upon SCF trigger, which occurs either by the binding of free ligand or by heterotypic cell-cell interactions, modulates different cellular, and molecular programs including stem/progenitor maintenance, differentiation, proliferation, and migration in hematopoietic (80), germ (81), melanocyte (82), and other lineages [(83, 84); Figure 1]. Several forms of unregulated cell growth and tumor development depend from c-kit activating mutations (85). Undifferentiated as well as terminally differentiated cell types, such as neurons (86), interstitial cells of Cajal (87), hematopoietic progenitor cells (88), mast cells (89), and Leydig cells and spermatogonia (90) express c-kit on their membrane. Furthermore, c-kit plays a key role in the development of most organ systems, particularly in pigmentation, hematopoiesis, oncogenesis, and reproduction (Figure 1). Worth noting here that c-kit is a vital gene as indeed c-kit deletion in homozygosis is incompatible with life.

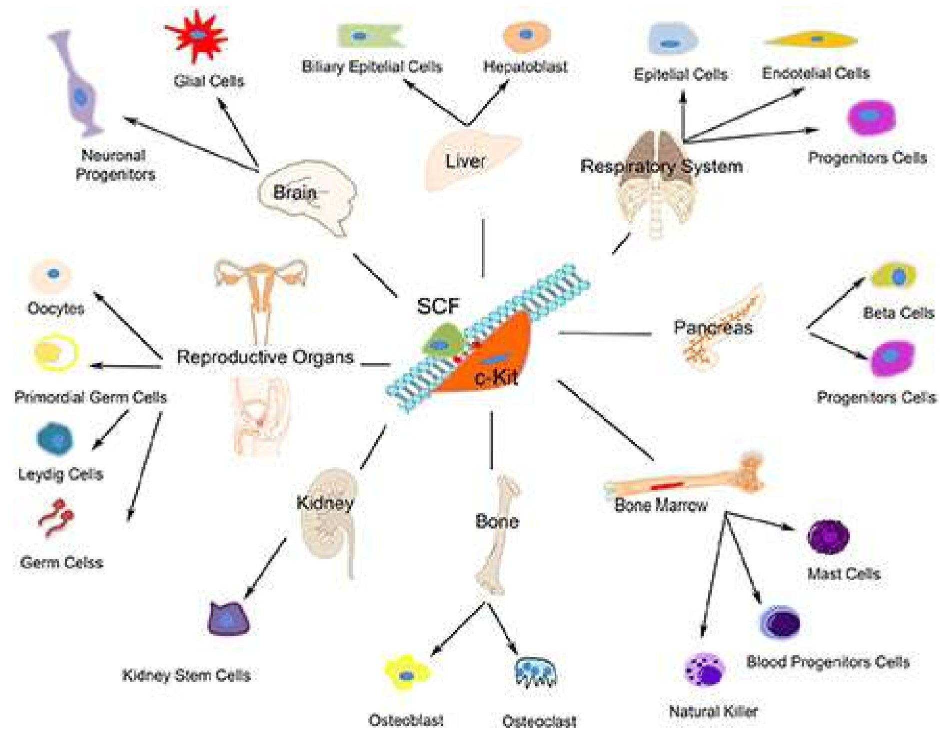

FIGURE 1. Schematic representation of c-kit expressing cells. The autophosphorylation of the c-kit receptor starts by the binding of its stem cell factor (SCF). As showed c-kit plays a significant role in the development of most organ systems such as neurons, interstitial cells of Cajal, hematopoietic progenitor cells, mast cells, Leydig cells, and spermatogonia.

In regards with heart biology, c-kit expression has been shown in embryonic life during heart cell specification and it has been shown to play a role adult heart repair after injury [(86, 91); Figure 2].

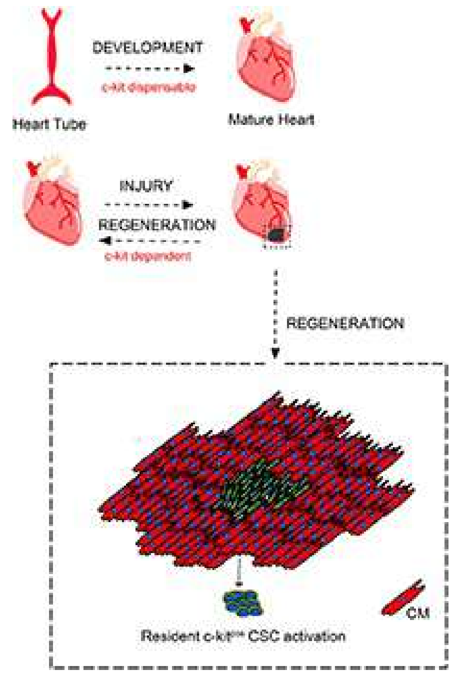

FIGURE 2. c-kit involvement in heart embryonic development and adult cardiac regeneration. During development c-kit expression appears dispensable for complete heart formation despite c-kit deletion is incompatible with life. Moreover, c-kit expression has been reported to be important also during heart cell specification and heart repair after injury through c-kit^{pos} CSCs activation. Adapted from Cianflone et al. (54) under the Creative Commons license CC BY-NC.

To follow c-kit expression in cardiac cells, Tallini et al. (40) generated a BAC transgenic mouse (c-kit^{BAC}-EGFP) in which the reporter gene, EGFP, is placed under the transcriptional control of the c-kit locus with the aim to have a reliable and easy-to-detect marker which maintains transcriptional fidelity. Using these c-kit^{BAC}-EGFP transgenic mice, it was demonstrated that c-kit expression marks *bona fide* cardiac progenitor cells in the neonatal heart. Neonatal c-kit^{pos}EGFP^{pos} cells are indeed able to differentiate *in vitro* into the cardiac cell lineages. Furthermore, when myocardial infarction is induced in these mice, a regenerative response is detected characterized by the recruitment of c-kit^{pos}EGFP^{pos} cells, neomyogenesis and neoangiogenesis. Accordingly, these data were confirmed in human samples whereby it was shown that c-kit^{pos} cells are more abundant in the right atrial appendage of neonates while they decrease within the first month of life (92–94).

Mice lacking the receptor tyrosine kinase c-kit have hematopoietic defects causing perinatal death (95–97) and defective c-kit signaling leads to compromised cardiac function (98, 99); Figure 2]. Spontaneous murine genetic mutations in the c-kit locus determine the so called White (W) phenotype and heterozygous W mutant mice have an impaired c-kit signaling that is associated with worsened cardiac remodeling after MI (91). On the other hand, transgenic mice with c-kit over-expression mount an improved reparative response after MI leading to an increased cardiac function (54, 100, 101).

Recently, the function of c-kit signaling in CSC biology and heart repair, has been investigated (102) through a transgenic mouse model carrying an activated c-kit mutation. In particular, cardiac tissue and cardiac cells derived from these transgenic mice are characterized by a constitutive activation of the c-kit receptor (54, 102), which exerts a protective and regenerative role in myocardial tissue after injury. Stable c-kit activation improves cardiac remodeling and repair after myocardial injury while it fosters proliferation and differentiation of eCSCs mainly through MAPK and AKT signaling activation (54). Indeed, ERK1/2 and AKT phosphorylation, the molecular effectors of c-kit receptor downstream signaling, are significantly increased in heart tissue as well as in CSCs from the "*c-kit-activated*" transgenic mice (54, 102). These signaling pathways are instrumental in the modulation of the activation and endothelial/myogenic differentiation of CSCs (54, 102). These data overall show that c-kit receptor signaling modulates CSC fate *in vivo* and CSC endothelial as well as cardiomyocyte differentiation follows c-kit receptor molecular activation (54, 102).

If on one hand the positive modulation of c-kit receptor function positively modulates CSC regenerative potential, on the other, a c-kit null allele, as the one produced by Cre recombinase insertion in the c-kit locus to develop c-kit^{Cre}-KI mice, offers a typical c-kit loss of function assay to assess whether eCSC function depends on an intact c-kit gene expression (54). Thus, we further tested the regenerative potential of Lin^{neg}c-kit^{pos} CSCs obtained from c-kit^{Cre}-KI mice compared with wild type Lin^{neg}c-kit^{pos} CSCs (wtCSCs) before *in vitro* and then transplanting them *in vivo* in a murine myocardial infarction model (54, 103). We confirmed that Cre knock-in induced a typical White (W) mutation in c-kit^{Cre}-KI mice that significantly reduced CSC proliferation and clonogenesis while nearly abolished the cardiomyogenic potential of these cells *in vitro* and *in vivo*. We then tested the effects of rescuing c-kit haploinsufficiency in c-kit^{Cre} CSCs by BAC-c-kit transgenesis. BAC-c-kit transfection normalized c-kit content in c-kit^{Cre} CSCs, which recovered a normal regenerative potential *in vitro* as well as *in vivo* after myocardial infarction (54, 103).

Therefore, the silencing of one c-kit allele, as resulting in a Cre knock-in mouse model, profoundly modifies c-kit biology and therefore this approach cannot be used to track c-kit expressing cells to study their physiology *in vivo*. An alternative approach to trace c-kit and evaluate its function in myocardial biology is the use of transgenesis to minimize all the deleterious effects of knock-in strategies. On this premise, Gude et al. generated a doxycycline-inducible transgenic mouse model to tag c-kit expressing cells with a long-lived, tetracycline responsive H2BEGFP (CKH2B) reporter (104, 105). In particular, they firstly confirmed that c-kit signaling promotes proliferation and survival of mouse and human cardiac progenitor cells while c-kit expression increases in response to cellular stress. The downstream effectors of c-kit phosphorylation, ERK and AKT, were coherently activated by SCF treatment and their activation was necessary for CPC activation *in vitro*. Furthermore, they compared the efficiency of identification of c-kit-labeled cardiac cells between the inducible-Cre knock-in line (c-Kit^{MCM}) and the c-kit^{H2BEGFP} transgenic model, analyzing myocardial tissue sections from these two mutant mice. Interestingly, they found a higher density of c-kit^{pos} cardiac cells in c-kit^{H2BEGFP} transgenic vs. c-Kit^{MCM} hearts further demonstrating the negative impact of the Cre knock-in in the c-kit locus that turns in a significant reduction of cardiac c-kit-expressing cells (104). Furthermore, EGFP tagging of c-kit^{pos} cardiac cells was higher in c-kit^{H2BEGFP} transgenic vs. c-Kit^{MCM} hearts (104).

Overall, these data underline that genetic reporter and fate track mouse models are imperfect reproductions of endogenous gene expression. Indeed, both employing an exogenous promoter segment or exploiting the endogenous gene via knock-in strategy have limitations and caveats to be taken into account and precisely controlled for (106). Transgenic promoter segments may lack important regulatory elements possibly favoring ectopic expression, while knock-in reporters often create a null allele of the gene of interest with potential serious consequences on target cells. Specifically, applying knock-in strategy for c-kit-expressing cell lineage tracing creates a null allele of the c-kit gene. Indeed, Cre knock-in site disrupts known regulatory elements in exon 1, thereby perturbing endogenous c-kit biology with significant consequences for stem cell function (107). Additionally, reporter expression constrained to one allele of the endogenous promoter, coupled with decreased c-kit function, as it is the case of Cre knock-ins produce decreased reporter sensitivity and consequent under representation of the tagged c-kit cell population (19, 103, 108).

Several studies reproduced the findings that c-kit signaling promotes growth, survival and proliferation in human CPCs *in vitro* (109), while W locus mouse mutants (W/Wv) exhibit c-kit cell dysfunction (110, 111). W/Wv mice indeed display impaired cardiac recovery after infarction (98), diminished cardiac function with advanced age (99), and compromised c-kit cell differentiation into cardiomyocytes (99, 112). Bone marrow ckit^{pos} cells from W locus mutants or cells in which c-kit has been molecularly silenced *in vitro* exhibit blunted reparative responses to myocardial injury (91, 98). Furthermore, the deletion of c-kit gene, as it occurs in homozygous W-mutated mice (113), causes murine premature death, because c-kit gene deletion is incompatible with life. However, c-kit-defective adult hearts appear to develop normally during embryonic life (48), while adult c-kit^{Cre}-KI mice have a significant defect in their regeneration potential after myocardial infarction *in vivo* (103). Therefore, it appears that c-kit plays divergent role in cardiac regeneration when compared to heart formation/development, which suggests that the molecular program underlying cardiac regeneration does not resemble cardiac generation. The latter is unpredicted when considering all the attempts currently ongoing to decode the pathways of developmental cardiac generation and neonatal heart regeneration to instruct effective protocols of adult cardiac regeneration (54).

Finally, the role of c-kit was evaluated in several models of cardiac pathology such as doxorubicin-induced cardiomyopathy (114–116), chronic heart failure (93, 117, 118), and aging cardiomyopathy (119, 120). In particular, Huang et al. developed a pediatric model of doxorubicin-induced cardiotoxicity in which juvenile mice were exposed to doxorubicin, using a cumulative dose that did not induce acute cardiotoxicity (114). These mice develop normally and have no obvious cardiac abnormalities as adults. However, these hearts have abnormal vasculature and a reduced number of c-kit^{pos} cardiac cells, which correlated with an increased sensitivity to physiological and pathological stimulus. When adult mice were subjected to myocardial infarction they developed a more pronounced cardiac decompensation, which correlated with a failure to increase capillary density in the injured area. Subsequently, it was demonstrated that the anthracycline-induced cardiomyopathy is caused by a depletion of functional c-kit^{pos} CSC pool and it can be rescued by restoring their function (115).

RESOLVING THE CONTROVERSY OVER THE ROLE AND MYOGENIC PROPERTIES OF THE C-KIT^{POS} CSCS

From the results summarized above, it was reasonable to expect that identification of the CSCs and characterization of their properties *in vitro* and *in vivo* would have put to rest any questions about the intrinsic regenerative capacity of the adult myocardium and about the origin of the CMs born in adulthood. Unfortunately, the notion that from a practical standpoint, the myocardium has neither intrinsic regenerative potential nor harbors tissue-specific stem cells with any meaningful myogenic capacities still persists (48, 121). This backwards view has persisted without a challenge to the reproducibility of published results which are the foundation of the new paradigm in heart biology (22, 32, 36, 37, 45, 52).

Putting aside the recent scandal over Anversa's group (see below), it remains the independently reproduced evidence arising from more than 15 years of scientific data (22). The burden of available scientific proof clearly shows that the adult mammalian heart harbors a pool of undifferentiated cells with cardiac regenerative potential, which are very small (5–7 μm in diameter), and are present in low abundance (1 CSCs per every (1–3) thousand CMs). Unsurprisingly, their identification, isolation and manipulation is naturally complex (53) as for the very nature of all adult tissue specific stem cells. *In vitro* and *in vivo* CSCs are *bona fide* myogenic progenitors, producing immature CMs of small size, which *in vitro* express cardiomyocyte-specific genes at levels similar to neonatal cardiomyocytes and *in vivo* undergo complete maturation over time, with terminal differentiation and permanent withdraw from the cell cycle (45, 52, 53).

On this premise, the detection in the healthy and pathological myocardium of a cohort of cells, which express myocyte-specific genes while still undergoing DNA replication should not be interpreted as evidence of adult cardiomyocyte un-expected division. Yet the sole identification of small mononucleated cells expressing CM-specific genes undergoing DNA replication and cytokinesis has been taken as sufficient proof that post-natal pre-exisiting cardiomyocyte division account for adult CM renewal, denying any contribution of CSC differentiation to new CM formation in adult cardiac tissue homeostasis and after injury (50, 122). Despite the latter, there is no confirmed evidence that mature and terminally differentiated CMs from any mammalian species can re-enter the cell cycle and undergo productive cytokinesis. All the so-called “pre-exisiting CM division” in adulthood occurs indeed in small-sized mononuclear CMs (122–124), while in rodents the vast majority of the adult CMs are mature-sized and bi-nucleated (125). Without any further evidence, the rare cases of cells expressing sarcomeric proteins while undergoing DNA replication and mitosis has recently been re-interpreted as evidence of a small pool of immature adult CMs which retain their proliferative competence (48, 122). This interpretation is based on the fact that in the neonatal life CMs, for a limited time window and before their terminal differentiation, can boost their replicative capacity and on the indisputable evidence that the CMs of certain fishes and amphibians are mitotically competent (126, 127). However, at present there is not a single piece of experimental evidence in support that these two phenomena have any relevance to CM renewal in the adult mammalian heart.

In contrast, no available data can dispute that the adult heart harbors resident CSCs and multiple laboratories have conclusively shown that these cells *in vitro* and *in vivo* generate *bona fide* cardiomyocytes together with vascular and connective tissue cells (30–35, 37, 45). Thus, the detection of dividing small, immature, and mono-nucleated CMs as found in the adult myocardium should be more appropriately interpreted as transient amplifying myocytes differentiated from a more resident stem/progenitor cell and surely not the proof of the division of pre-existing CMs (20, 128).

Recently several genetic murine approaches to track *in vivo* the so-called “c-kit^{pos} cardiac cells” (28, 29, 48–51, 121, 129) has generated a significant confusion calling for “a re-evaluation of the real myogenic potential of the cardiac c-kit^{pos} CSCs” (48).

Using either c-kit^{Cre}-KI mice or c-kit^{CreER}-KI mice, these authors reported that “cardiac c-kit^{pos} cells” mainly differentiate into endothelial cells and minimally, if not negligibly, contribute CMs either in neonatal or adult life, or after injury (48–50). According to these findings, it was claimed that the “cardiac c-kit^{pos} cells” are not CSCs at all but just endothelial committed cells (48, 49). Moreover, the regenerative potential of “cardiac c-kit^{pos} cells” is limited to neoangiogenesis and to cardiac interstitial cell formation (54).

To critically analyze the findings of these reports, it must be first remembered, as discussed above, that c-kit^{pos} cardiac cells are a heterogeneous cell population whereby in the adult heart >90% of c-kit^{pos} cells are mast cell/endothelial lineage-committed cells. Genetic fate mapping strategy base on the Cre-lox recombination system, nowadays considered “the gold standard” to address the exact regenerative potential of a given cell population, has been then customized to track the fate of c-kit-expressing cells *in vivo* (48–50). The latter was deemed sufficient by the proposed experimental design to include also c-kit-expressing CSCs. Unfortunately, all the insertions and deletions required to introduce the Cre recombinase into the c-kit locus have resulted in a null c-kit mutation, which does not produce the corresponding mRNA (103). Thus, these mice could be used only in heterozygosis while carrying a significant genetic defect with physiological consequences. Cre recombinase detects DNA sequences flanked by a specific 34-bp sequence called loxP removing the flanked sequence, and leaving single loxP site in place (130–133). This technology is used to delete a transcriptional stop sequence such that a reporter gene starts to be expressed after Cre recombination. The latter is the basis of the "indelible labeling" by Cre-lox-based lineage-tracing experiments. In these experiments, DNA excision at loxP sites is dependent on Cre expression whereby recombination occurs only in those cells that express or had expressed Cre recombinase. By placing the Cre cassette under the control of a specific gene promoter, recombination is directed to a particular cell expressing that particular gene. However, the mapping system is guided by Cre levels, whereby recombination efficiency is proportional to Cre levels. This is crucial because despite two cell types express the Cre-targeted gene but at different levels, not necessarily the two cell types will be equally recombined (54). If Cre levels efficiently recombine only one of the two cell types, the resultant fate map will underestimate the descendant population of the un-recombined cell type (54). Accordingly, the cells with lowest expression of Cre, because of the low expression of the Cre-engineered gene might fail to have their fate tracked (54, 133). In the case of c-kit fate tracking experiments, the Cre-dependent recombination efficiency is directly proportional to the level of Cre expression from the null c-kit allele (19, 133, 134). Considering that most stem cell types express low level of c-kit (54, 103, 135), and particularly c-kit^{pos} CSCs (54, 103), it was highly questionable whether the null c-kit^{Cre} allele could recombine a meaningful fraction of the c-kit^{pos} CSCs to track their fate (19).

Indeed, Vicinanza et al. (103) and Cianflone et al. (54) have recently shown that c-kit expression level in adult CSCs is low and the c-kit^{Cre} allele in c-kit^{Cre} KI mice produces insufficient amounts of Cre to effectively recombine the floxed Cre-reporter gene to tag the CSCs and fate their progeny (54, 103). Thus, c-kit^{Cre}-KI models (48, 49) only minimally, if not negligibly, tag, and fate map resident CSCs. Furthermore, Cre-KI into c-kit locus in all cases has produced a null c-kit allele that fatally impairs *in vitro* and *in vivo* CSC properties (54, 103). This non-physiologic and inefficient recombination system, produced by the c-kit^{Cre}-KI model, determines a very low number of c-kit^{pos} progenitor-generated cardiomyocytes detected in c-Kit^{cre} mice. This picture reflects the failure to recombine the CSCs to track their progeny and the severe defect in CSC myogenesis produced by the c-kit^{cre} allele (54, 103). For these reasons, unavoidably, all the c-kit^{Cre} knock-in mice show a scant CM progeny *in vivo* during homeostasis and after injury (48–50). Astonishingly, despite lacking proper controls (19) and despite the evidence for their severe limitations (103), the results arising from c-kit^{Cre} KI mice have been taken as evidence that c-kit^{pos} CSCs do not exist or have a marginal myogenic regenerative potential (136).

Overall, while these papers have been proven wrong and unreliable, they have generated significant and unnecessary upheaval in the cardiac repair/regeneration field (19, 20).

To correctly track the fate of endogenous CSCs requires a c-kit-driven Cre KI mouse model that does not affect c-kit expression and in which Cre is produced in amounts sufficient to recombine the marker gene. Li et al. (137) attempted to overcome this problem using a dual reporter in which two loxP sites were interleaved so that either a Dre-rox or Cre-loxP recombination would remove the substrate of the other resulting in the permanent tagging of the cell. This system was used to label “all” CMs and non-CMs with two different markers. Surprisingly, they only ascertained that the “majority” of the cells were labeled but never tested whether this system was tagging the CSCs. Therefore, despite the controversy, they overlooked a basic rule of cell-fate tracking, which is that the marker used has to effectively tag the cell which fate is to be tracked. To assert that the system used tags most “non-myocyte” cells means very little, particularly when the population to be tracked (the cardiac stem/progenitor cells) represent <1% of the non-myocyte population which is labeled. Therefore, the main issue in the paper by Li et al. is that the authors never tested whether cardiac stem/progenitor cells were indeed labeled among the non-myocyte population. The authors did not carry out this essential step. Furthermore, another issue with Li et al. paper is that because they never tested if and how the adult CSCs were labeled, they cannot exclude the hypothesis that they were instead labeled by the myogenic/myocyte promoters they used in embryo life. The latter is a key step because it is known and proved that the Tnn2 promoter (used by Li et al.) during heart development in embryonic life not only labels cardiomyocytes but also endothelial cells covering aortic and pulmonary valves (138), a fact that indirectly shows that Tnn2 promoter activity in embryonic life labels multipotent cardiac progenitors. Additionally, adult cardiac stem/progenitor cells express Tnn2 mRNA *in vivo* (139, 140).

In short, despite contradictory publications, any objective review of the data shows that the eCSCs are genuine cardiac stem/progenitor cells and the main, if not the only, *bona fide* source of new cardiomyocyte formation in the healthy and pathological adult heart.

CONCLUSIONS

The long-standing paradigm of the heart as a non-regenerative organ has been replaced by a wealth of data showing that new cardiomyocyte (CMs) are formed throughout life and after injury in the adult mammalian heart. It is also clear, however, that this regeneration on its own is not robust enough to repair severe segmental myocardial damage such as post-AMI, the main cause of HF. Overall, the data available show that when correctly identified and expanded the endogenous CSCs are robustly myogenic *in vitro* and *in vivo*. Unfortunately, despite this reproducible evidence, some recent work has questioned what was a growing consensus about the origin, quantity, and physiological significance of the CMs generated in adulthood in response to wear and tear and/or injury, which has severely muddled the field of adult cardiac regenerative biology. To move forward past this controversy is a crucial step for the adult cardiac regenerative biology field.

Very sadly and unfortunately, the recent scandal that brought to the request for and the retraction of a significant number of papers from the group of Piero Anversa, a group that historically was among the first to contribute to the discovery and characterization of adult cardiac progenitors, has created a “tsunami” for the field of adult cardiac stem cell biology (136). Clearly, scientific misconduct is a very serious issue which needs to be dealt seriously but honestly. It is understandable also that what has become public is sufficient reason to critically review Anversa's publications and the data which have been manipulated should be immediately retracted. However, it is mandatory that institutions, journals, and the scientific establishment in general will do that objectively and using the same parameters applied to others who have had publications retracted and/or discredited. Instead and very sadly, some investigators are using this turbulence to discredit all work related to myocardial repair/regeneration and cardiac stem cells, even though a significant part of the work which they now assail, has no relation to and was not authored by Anversa's group. Ironically, while arguing that, based on the revelations as to Anversa, all papers about myocardial repair/regeneration based on myocardial stem cells should be either ignored or retracted, these investigators fail to point out that the work providing negative data on this subject has been found incorrect and the relative methodology and conclusions have been shown to be invalid (103). In short, to call into a ban all the independent work produced by the cardiac stem cell field just on the basis of the proven and alleged Anversa's misdeeds is as unscientific as these misdeeds are.

It is clear that the c-kit^{Cre}-KI strategies for CSC identification and cell-fate mapping have such severe limitations as to make them unsuitable for either the identification or fate-map the c-kit^{pos} CSCs. The very low number of endogenous c-kit^{pos}CSC-generated cardiomyocytes detected in the c-kit^{Cre} mice does not reflect a minimal myogenic potential of the CSCs but it simply reflects the failure of the KI-Cre to recombine the CSCs to track their progeny together with the severe defect in CSC myogenesis produced by the c-kit^{Cre} null allele.

When the pitfalls of the c-kit^{Cre}-KI are taken at a face value it follows that the results of the experimental approach though endogenous CSC ablation and their exogenous replacements clearly stand and indisputably show that the CSCs are necessary and sufficient for robust cardiomyogenesis and to support myocardial regeneration/repair in response to diverse types of damage. This phenotype requires and is dependent upon a diploid level of c-kit expression. Confirmation of these conclusions using novel and reliable genetic fate map strategies should clear the way for the potential development of CSC-based myocardial regenerative protocols.

AUTHOR CONTRIBUTIONS

DT, FM, VM, and BN-G have contributed to the conception or design of the work. MS, EC, TM, IA, VA, MT, and DP contributed to the acquisition, analysis, or interpretation of the data for the work. DT, FM, and BN-G drafted the work and revised it critically for important intellectual content. MS, EC, TM, IA, VA, DP, MT, and VM revised the work critically for important intellectual content. All the authors gave their final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REVIEW
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Addressing Vulvovaginal Atrophy (VVA)/Genitourinary Syndrome of Menopause (GSM) for Healthy Aging in Women

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Vaginal health is an essential component of active and healthy aging in women at midlife and beyond. As a consequence of hormonal deprivation and senescence, the anatomy and function of urogenital tissues are significantly affected and vulvovaginal atrophy (VVA) may occur. In a high proportion of postmenopausal women, progressive and chronic VVA symptoms have a strong impact on sexual function and quality of life. The new definition of genitourinary syndrome of menopause (GSM) comprises genital symptoms (dryness, burning, itching, irritation, bleeding), sexual symptoms (dyspareunia and other sexual dysfunctions) and urinary symptoms (dysuria, frequency, urgency, recurrent urinary infections). Many variables (age, sexual activity and partnership status) influence the clinical impact VVA/GSM symptoms and attitudes of elderly women to consult for receiving effective treatments. Psychosocial factors play a critical role in sexual functioning, but the integrity of the urogenital system is as well important affecting many domains of postmenopausal women's health, including sexual function. Several international surveys have extensively documented the need to improve VVA/GSM management because of the strong impact on women's daily life and on couple's intimacy. Health care providers (HCPs) need to be proactive in the early recognition of VVA/GSM in order to preserve urogenital and sexual longevity, by using hormonal and non-hormonal strategies. The clinical diagnosis is based on genital examination to identify objective signs and on the use of subjective scales to rate most bothersome symptoms (MBS), especially vaginal dryness. Recent studies point to the importance of addressing VVA/GSM as a potential early marker of poor general health in analogy with vasomotor symptoms. Therefore, a standard of VVA/GSM care in elderly women is desirable to enhance physical, emotional and mental well-being.

Keywords: vulvovaginal atrophy (VVA), genito-urinary syndrome of menopause (GSM), aging, longevity, vaginal dryness, dyspareunia, female sexual dysfunction (FSD), quality of life (QoL)

INTRODUCTION

Women live longer than men all around the world (1) and in developed countries they expect to survive more than 30 years following natural menopause, which usually occurs between 48 and 52 of age (2). That being so, the impact of reproductive aging on healthy longevity becomes increasingly important because of the potential conditions associated with menopause-related hormonal deficiency (3). Estrogen deprivation is the hallmark of ovarian exhaustion leading to the manifestation of several signs and symptoms with a significant impact on quality of life (QoL) and on physical, mental and sexual health (4). Even androgen insufficiency, an endocrine feature more evident in women with premature ovarian failure (natural, surgical, iatrogenic), may contribute to the clinical events related to menopause (5). Separating the effect of menopause from the variety of changes associated with senescence is quite difficult, but recent observations bring about the idea that menopause accelerates biological aging, especially when reproductive failure occurs prematurely (6).

The present narrative review points to the importance of addressing the chronic condition of vulvovaginal atrophy (VVA)/genitourinary syndrome of menopause (GSM) in the context of promoting urogenital and sexual longevity in women at midlife and beyond. It merely reflects the expert opinion of the authors by analyzing the amount of available evidence (1990–2019) in this complex field of research. Therapeutic strategies to effectively manage sexual symptoms associated with VVA/GSM have been reviewed extensively elsewhere (7–12) and, in here, they will be discussed briefly to serve the scope of preventing severe VVA/GSM in elderly women.

MENOPAUSE AND UROGENITAL AGING

Among the multitude of menopausal complaints, vasomotor symptoms (hot flushes and cold or night sweats) and vaginal dryness have clearly shown a strong relationship with low estrogens during and after the menopausal transition (13). Up to 80% of women experience vasomotor symptoms during menopause with an average duration of 10 years and a variable degree of severity (14). Untreated vasomotor symptoms may represent a biomarker of chronic postmenopausal conditions such as cardiovascular disorders and osteoporosis (15). However, they do not usually progress over time (16) and remain problematic for a lower number of postmenopausal women aged 60–65 years (17). Unlike vasomotor symptoms, vaginal dryness is highly present also in older women because it is the cardinal symptom of vulvovaginal atrophy (VVA) (18), a chronic condition starting around menopause, mainly as a consequence of estrogen deficiency (19), and progressing with chronological aging and medical morbidity (20). The majority of postmenopausal women have signs of VVA upon physical examination, especially if they consult for vaginal dryness (21), but less than half of the postmenopausal population report VVA symptoms as bothersome in international surveys (22–25). There is a lack of understanding surrounding vagina health (26) and elderly women do not discuss VVA symptoms so easily because sexual health is a sensitive topic (27). In addition, the condition is believed to be transient and part of the natural aging phenomena (28, 29). In the Vaginal Health: Insights, Views & Attitudes (VIVA) survey, 55% of women with vaginal discomfort reported experiencing symptoms for 3 years or longer and only a minority (4%) attributed their symptoms to vaginal atrophy (25). Age, attitudes toward menopause, sexual activity, chronic disorders, previous and/or current use of menopausal hormone therapy and other biopsychosocial determinants influence the level of distress associated with VVA symptoms and the rate of reporting female sexual dysfunction (FSD) (30, 31). General and sexual health of the partner, as well as the quality and duration of the relationship, are also very important and addressing age-related changes in both members of a couple may contribute to a better management of VVA and sexual dysfunctions (32).

Urogenital aging is an old problem, newly recognized, which can be highly prevented upon early recognition of signs and symptoms (33). Vaginal dryness, followed by dyspareunia, is the most common symptoms reported by postmenopausal women both in surveys (22) and in clinical studies (21, 34). In the REVIVE surveys conducted both in United States (US) (23) and in Europe (EU) (24) the onset of VVA symptoms has already been reported in the majority of women within the perimenopause/early postmenopause. Interestingly, in the AGATA study, which included a sample of Italian women asking for a routine gynecological examination, a clinical diagnosis of VVA displayed a prevalence ranging from 64.7 to 84.2%, starting from 1 to 6 years after menopause (35). It is essential that health care providers (HCPs) are proactive to uncover the topic of vaginal health because women who discuss VVA with HCPs are twice as likely to be current specific-treatment users (59.7% as compared to 22.7% who did not discuss VVA) (28). It is frequent to encounter a disconnection in education, communication, and information between HCPs and their menopausal patients (36). The WISDOM survey outlined that the comfort level of HCPs when prescribing VVA treatment is still suboptimal, in particular in case they are not gynecologists (37). Education of women, adequate training of HCPs and provision of communication tools in order to facilitate the “uncomfortable” dialogue are potential solutions to address the barriers currently impeding patient–clinician interactions around sexual health (38).

Basic counseling is the first step in the management of postmenopausal sexual dysfunctions (39) and a standard process of care developed by the International Society for the Study of Women's Sexual Health (ISSWSH) may provide guidance to HCPs to effectively recognize sexual concerns and problems in women (40).

VULVOVAGINAL ATROPHY (VVA) OR GENITOURINARY SYNDROME OF MENOPAUSE (GSM): WHAT IS IN THESE TWO NAMES?

In recent years, VVA has a new name, genitourinary syndrome of menopause (GSM), to underline the multitude of genital, sexual and urinary symptoms associated with the anatomical and functional changes of vulvo-vaginal tissues occurring with menopause and aging (41). A terminology consensus conference cosponsored by the North American Menopause Society (NAMS) and by ISSWSH was held in May 2013 to review the most relevant scientific literature in the field of postmenopausal urogenital and sexual health. Following a 2-day discussion, acknowledged experts agreed on the need of having a new term to describe more accurately the condition previously known as VVA. The choice of GSM was the result of many considerations, including the need of a term more acceptable in the medical and public arena to improve and increase communication, research, education and management of urogenital and sexual symptoms in postmenopausal women. The definition of syndrome is used to describe a collection of clinical signs and symptoms (genitourinary) correlated with each other, that do not have to be all present and related to a single identifiable pathogenesis, but occur in a particular circumstance (menopause). That being so, GSM is defined as “a collection of symptoms and signs associated with a decrease in estrogen and other sex steroids involving changes to the labia majora/minora, clitoris, vestibule/introitus, vagina, urethra, and bladder. The syndrome may include but is not limited to genital symptoms of dryness, burning, and irritation; sexual symptoms of lack of lubrication, discomfort or pain, and impaired function; and urinary symptoms of urgency, dysuria, and recurrent urinary tract infections (Table 1). Women may present with some or all of the signs and symptoms, which must be bothersome and should not be better accounted for by another diagnosis” (41).

Table 1. Most common subjective and objective symptoms to diagnose vulvovaginal atrophy (VVA)/genitourinary syndrome of menopause (GSM) in daily practice.

Subjective symptoms (0 = none; 1 = mild; 2 = moderate; 3 = severe; not applicable = N/A symptoms related to sexual activity)
Vaginal Dryness
Dyspareunia
Irritation/Burning/Itching
Dysuria
Bleeding with sexual activity
Objective signs (clinical scale: 0 = normal; 1 = mild; 2 = moderate; 3 = severe)
Elasticity
Vaginal folds
Fluid secretion
Epithelial thickness
Moisture
Color of the tissues

VVA is strictly related to estrogen deficiency and is an integral part of GSM (10). However, the new definition GSM includes signs and symptoms that cannot be all reversed by estrogen replacement and may require different strategies according to their true etiology (42). As examples, vulvar dermatological conditions (43), vulvodynia (44), and pelvic floor dysfunction (45) have an increased prevalence in postmenopausal women, may co-occur with VVA, but have their own specific treatment protocols. At present, the majority of data were published with available questionnaires and scales validated to identify VVA-associated signs and symptoms and further studies are need to fully understand the multitude of disturbances included in the GSM definition. Recently, a novel patient-reported outcome measure exploring experiences of women with GSM was designed for use in both clinical care and research (46). The hope is to gain new insight into the biopsychosocial determinants of GSM in order to tailor evidence-based treatments for the individual woman across different stages of post reproductive lifespan.

PHYSIO-PATHOLOGICAL ASPECTS OF VVA/GSM

Hormonal fluctuations driving the female reproductive life cycle highly modulate the functional anatomy of the uro-genital and pelvic tract. Early data showed that untreated postmenopausal women displaying <50 pg/ml of circulating estradiol suffer more from symptoms associated with VVA (47). A historical study (the only citation prior 1990) demonstrated that even endogenous androgens may play a role because objective signs of VVA were less evident in postmenopausal women with significantly higher mean levels of androgens (androstenedione and testosterone) and gonadotropins (particularly LH). These women were more sexually active (intercourse frequency, three or more times monthly) as opposed to the sexually inactive women (intercourse frequency, <10 times yearly) (48). Whether stronger sexual desire and responsiveness driven by androgens protected against VVA or, alternatively, androgens had a direct action on peripheral tissues was not established by the "the use it or lose it" theory. However, these data are in line with the evidence that both circulating estradiol and its androgen precursors (dehydroepiandrosterone/dehydroepiandrosteronesulphate [DHEA/DHEAS], androstenedione, testosterone), as well as their local metabolites, are vital to maintain normal structure and function of the vagina and surrounding uro-genital tissues (49). Indeed, the science of intracrinology supports the idea that the age-related decline of circulating DHEA translates into a local intracellular deficiency of both estrogens and androgens, significantly contributing to poor vaginal health (50). During reproductive life, the vagina, vulva, pelvic floor muscles, endopelvic fascia, urethra, and bladder trigone display a significant amount of estrogen receptors (ERs, both α and β), which decline with menopause and may be restored by the use of systemic and local estrogen treatment. ERs are mainly expressed in the epithelium and in stromal and muscle cells of the human vagina. Even androgen receptors (ARs) are largely expressed at multiple levels (mucosa, submucosa, stroma, smooth muscles, and vascular endothelium) and cross-talk with ERs, influencing neurovascular and neuromuscular function under different endocrine conditions (51). Estradiol controls a plethora of cellular pathways regulating growth and proliferation, barrier function and pathogen defense (52). The main consequence of lacking estrogen stimulation is the loss of tissue elasticity by inducing fusion and hyalinization of collagen fibers and fragmentation of elastin fibers. The mucosa of the vagina, introitus, and labia minora becomes thin and pale and appears less hydrated. The vaginal canal becomes shorter and narrow because the vaginal rugae, the epithelial folds that allow for distensibility, progressively disappear. In addition, there is significant reduction of vascular support leading to a decrease of the volume of vaginal transudate and of other glandular secretions (53). Both estrogens and androgens contribute to pelvic nerve-stimulated genital blood flow, tissue response to neurotransmitters and sensory threshold to stimuli

(51). Over time, intercellular acid mucopolysaccharide and hyaluronic acid are significantly reduced in the dermal layer. Moreover, there is a progressive dominance of parabasal cells with fewer intermediate and superficial cells. This means the vaginal squamous epithelium is quite completely estrogen deprived. Therefore, it becomes friable with petechiae, ulcerations, and eventually bleeding after minimal trauma (54–61). A thinner vaginal epithelium is also associated with a significant reduction of glycogen which translates into a lower amount of lactobacilli causing an increase in vaginal pH (between 5.0 and 7.5). The subsequent decrease of vaginal hydrogen peroxide allows the growth of other pathogenic bacteria (staphylococci, group B streptococci, and coliforms) causing atrophic vaginitis, vaginal discharge and odor. Indeed, lactobacilli diversity and abundance significantly decreased following menopause (62) and the vaginal microbiota of women with mild or moderate atrophy had a distinct bacterial community state, which may predispose to develop vaginitis and other uro-genital infections (63).

The neurovascular and neuromuscular substrates of the pelvic area are also impaired because the vulva, as well as the pelvic floor and the urinary tract, manifest similar anatomical and functional changes (64–66). In particular, entry dyspareunia, irritation, burning and itching of external genitals may be the result of the stenosis of the vulvar introitus. Indeed, hymeneal carunculae and the vestibule display less elasticity and the urethral meatus appears prominent and more vulnerable to trauma. Several changes of the urinary system (reduced urethral closure pressure, reduced sensory threshold in the bladder, and, in some cases, increased risk of rUTIs) may be observed as a consequence of the thinning of the urinary epithelium and weakening of the surrounding tissue (53).

KEY-ELEMENTS OF VVA/GSM DIAGNOSIS

Clinical interviews and rating scales to score the most bothersome symptoms (MBS) (Table 1) are useful instruments to measure subjective symptoms and to identify risk factors for VVA/GSM. Objective diagnosis is confirmed by an accurate pelvic examination, including gentle inspection of the vulva, vestibule, vagina, and urethra in order to recognize the signs of VVA/GSM (Table 1) which can be rated on validated scales (67). The Vaginal Health Index Score is a clinical tool that, by evaluating 5 parameters (vaginal elasticity, vaginal secretions, pH, epithelial mucous membrane, vaginal hydration), allows to obtain a final score defining the degree of atrophy in the genitourinary tract by assigning a single score to each parameter. Total score ranges from 5 to 25, with lower scores corresponding to greater urogenital atrophy (68). Vulva Health Index evaluates labia, urethra, clitoris, introitus as well as elasticity and pain during intercourse; total score ranges from 0 to 24, with higher scores corresponding with greater vulvar atrophy. If the Vulva Health Index is over 8 or there is score of 3 (severe) in any category, vulvar atrophy is suggested (69). In the most severe cases, tissues may be easily traumatized and irritated by touching or inserting the speculum (70). Organ prolapse or hypertonicity of the pelvic floor with secondary vaginism may be also present, as well as vulvovaginal signs which require a differential diagnosis by performing colposcopy or carrying out bacteriological analyses (11). In general, VVA/GSM is typically a clinical diagnosis and few laboratory tests may be used to support the evidence. Among them, the evaluation of vaginal pH and the vaginal maturation index (VMI) are the most used (41). With the VMI it is possible to identify the relative proportion of parabasal, intermediate, and superficial vaginal epithelial cells. Hypoestrogenism and atrophy are suggested when there is a dominance of parabasal cells, calculated on specimens obtained directly from the lateral upper vaginal walls. Thus, the shift to a higher number of superficial cells is a primary end-point of any treatments prescribed to relieve symptoms of VVA (71). Even, vaginal pH alone is a simple outpatient procedure, influenced by infections and intimate products, which reflects the hormonal milieu and its effects on the vaginal epithelium. Indeed, it consistently correlated with parabasal and superficial cells and the visual vaginal epithelial changes and symptoms of dryness and dyspareunia (72).

In both clinical and research settings, subjective assessment (the MBS approach) and objective assessments of VVA (measurement of vaginal maturation index and vaginal pH) should be combined according to a recent systematic literature search (73). Even though a high rate of subjective symptoms is associated with a clinical diagnosis of VVA/GSM in over 90% of the cases (21), objective signs and subjective symptoms have a different prevalence distribution in the years after menopause and are not strictly associated (35). However, self-reported and visible vaginal dryness do correlate and together with ph> 5, mucosal pallor, and rugae thinning seem to be the most important objective signs to make a diagnosis (35). On the other hand, the presence of other vulvar and urinary signs are relevant to the severity of VVS/GSM and its impact on women's daily living (74).

Notwithstanding these findings, HCPs may pose very simple questions to facilitate an open conversation on urogenital health and to record the variety of vaginal, vulvar and urinary symptoms. Visual vaginal, vulvar and pelvic assessment by HCPs is a useful measure for diagnosing VVA/GSM and assessing response to treatment. Moreover, it may help HCPs to identify women at risk of vaginal dryness and dyspareunia, and allow them to proactively engage in conversations about sexual health (75). Figure 1 reports a very simple check-list to diagnose VVA/GSM in routine clinical practice.

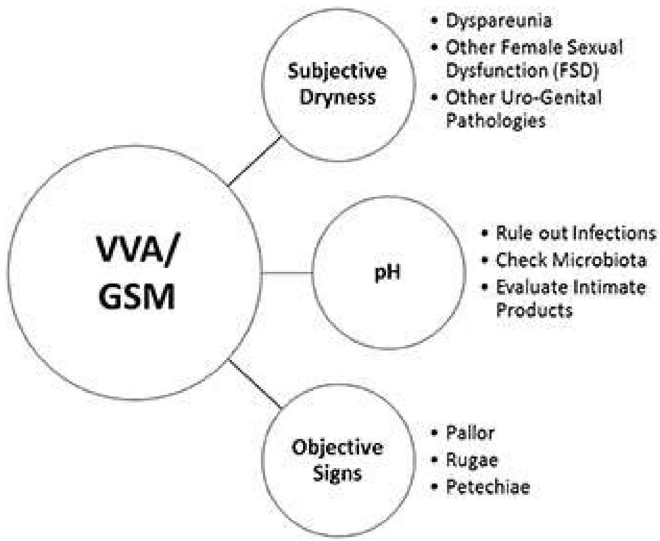

FIGURE 1. A very simple check-list to diagnose VVA/GSM in routine clinical practice.

Women with breast cancer and other gynecological malignancies are at very high risk of VVA and associated symptoms. Indeed, endocrine chemotherapy, surgery and/or radiation may induce profound changes at urogenital levels which have to be timely recognized in the oncologic care (76, 77). Moreover, we lack data on VVA/GSM in women with spontaneous premature ovarian insufficiency, even though it is likely that the condition is more distressing due to the younger age of these patients (78). Older women and those who abstain from sexual activity may suffer even more of VVA/GSM with vaginal and introital stenosis, fusion of the labia minora to the labia majora, and other urogenital conditions (79). Preventive gynecology is significantly challenged by the presence of severe VVA/GSM. Indeed, it may be difficult to adequately assess both cytologic and colposcopic findings to prevent cervical cancer. On the other hand, an episode of postmenopausal bleeding, very common in women with VVA/GSM, may cause an urgent referral to exclude endometrial cancer and other malignancies. Finally, even if less common, an early diagnosis of cancer may be delayed by vaginal synechiae and hematocolpos due to vaginal occlusion (80–82).

THE BURDEN OF VVA/GSM ON WOMEN'S SEXUAL FUNCTION AND QUALITY OF LIFE (QOL)

In the last decade, many international surveys attempted to clarify the impact of VVA/GSM on sexual function and QoL (Table 2) indicating that a proactive approach to conversations about vulvovaginal discomfort would improve diagnosis and treatment (22). Even though the proportion of women who are sexually active decreases with advancing age, the value of discussions about sexual health is still high in elderly women who are in partnership (83). In the survey of Midlife Development in the United States (MIDUS II) women who were married or cohabitating had approximately 8 times higher odds of being sexually active, with more than 30% of women over 65 years reporting sexual activity at least once a week (84). Sexual satisfaction is highly dependent on many psychosocial aspects related to well-being (85). In addition, dimensions of sexual response are part of the domino effect of menopausal symptoms, including weight gain, depression, anxiety and poor physical health (86). VVA/GSM is a clear medical condition that can be associated with impairment of sexual activity and intimacy within couples at menopause (19). VVA symptoms have an approximately linear relationship with sexual functioning (87) and VVA correlates with sexual inactivity in the Hormone Therapy (HT) Trials of the Women's Health Initiative (WHI) (88). These findings are in contrast with an early study showing in a little sample of pre- and postmenopausal women that current sexual activity was not associated with differences in vaginal length or introital caliber (89). On the other hand, the international CLOSER survey investigated the impact of VVA on postmenopausal women and on male partners demonstrating that intimacy avoidance was attributed to painful sex by a significant proportion of women (55%) and men (61%) (90). That being so, the assessment of sexual well-being at menopause should rule out not only the clinical signs of VVA/GSM but also the multitude of aspects associated with it, especially hypoactive sexual desire disorder (HSDD) which is a strong determinant of maintaining sexual activity and emotional intimacy within the relationship (91).

Table 2. Most common dimensions affected by vulvovaginal atrophy (VVA)/genitourinary syndrome of menopause (GSM) in international surveys.

Sexual dimensions
Satisfaction
Intimacy
Spontaneity
Loving relationship
Sexual activity
Quality of life dimensions
Sleep
Enjoinment
Sportive activity
Work/social activity
Feminine role
Sense of youth

Cultural aspects are strongly related to the interpretation of results from surveys on VVA/GSM and explain differences in reporting bothersome symptoms and consequences associated with them. For example, women reporting VVA in Southern Europe stopped having sex in 18 % of the cases (92), in Northern Europe in 22% (92), in UK in 27% (93) and in North America (US and Canada) in 29% (94). In addition, both US and EU REVIVE surveys underlined the strong impact of VVA on sexual satisfaction and sexual spontaneity, as well as on intimacy and relationship with the partner (23, 24). Of interest, EU participants acknowledged a significantly higher impact of VVA symptoms on sexual intercourse and partner interaction than US participants, and both cohorts were observed to have differences between their respective VVA symptom profiles (95). Apart cultural attitudes in the health care system or in the importance to maintain sexual activity over time, other elements of difference may be found between US and EU samples at baseline, including age, marital status, education, and working activity (95). Indeed, other studies indicate that the true prevalence of each symptom and the rate of distress associated with it are significantly influenced by many factors, namely age and sexual activity (96, 97). Dyspareunia is generally less reported later in life mainly because older women are less likely to still have a spousal or other intimate relationship (83). Behavioral profiles of postmenopausal women play also a role in disclosing VVA symptomatology and actively seeking treatment (98). Data collected in the CLOSER survey indicated that the VVA condition related to many dimensions of womanhood, in particular perception of aging and poor health (90, 99). In the “women's voices in the menopause” survey (27), 52% of respondents with vaginal discomfort reported an impact on their QoL. Both VIVA and CLOSER international surveys further explored the dimension affected by self-reported VVA symptoms demonstrating an influence on working, social activity and other aspects of personal well-being (24, 25). In addition, other data indicated that VVA is associated with a clinically significant impact on QoL that may be comparable to that seen in serious conditions such as arthritis, chronic obstructive pulmonary disease, asthma and irritable bowel syndrome (100, 101).

The EVES study collected very accurate information in a clinical population of EU (Italy and Spain) postmenopausal women aged 45–75 years reporting at least one subjective VVA symptom and objectively diagnosed with VVA during gynecological examination. Women scored 19 potentially VVA-related complaints on a 4-point severity scale (absent, mild, moderate and severe) and filled in both the EuroQol questionnaire (EQ-5D-3L) (102) and the Day-to-Day Impact of Vaginal Aging (DIVA) questionnaire to measure the impact of VVA on several dimensions of QoL (103). Sexual function and distress were also evaluated by validated questionnaires (104, 105). During gynecological clinical assessment, signs of VVA were rated in order to calculate the Vaginal Health Index (68) and the Vulva Health Index (69). The main outcomes of EVES showed that of a total of 2,160 evaluable women, 66.3, 30.5, and 11.2% suffered from severe vaginal, vulvar, and urinary symptoms, respectively. VVA was confirmed in more than 90% of the participants. Both generic and vaginal aging-related QoL scores showed a significant relationship with the different types of severe VVA symptoms. QoL questionnaires displayed worse scores in women where the diagnosis of VVA was confirmed by gynecologic examination. The severity of urinary symptoms showed a more strong impact on all DIVA components (daily activities, emotional well-being, sexual functioning and self-concept/body image) compared to vaginal and vulvar symptoms (74). This data confirmed recently reported observations on predictors of impact of vaginal symptoms, in which women with urinary incontinence reported a higher impact of VVA symptoms on three of the four DIVA dimensions (not sexual functioning) (106). In the Italian subset of 1,226 postmenopausal women, those with objective confirmation of VVA had worsened sexual function and distress when compared with the patients having only subjective VVA symptoms (107). Interestingly enough, postmenopausal women with VVA receiving treatment complained of more severe symptoms than those untreated. Moreover, time since menopause was significantly higher in women treated for VVA.

Collectively, EVES data indicate that VVA treatments should ideally be initiated at a younger age when symptoms commence and cause distress, before the condition becomes very severe and difficult to be reverted (108).

GENERAL PRINCIPLES FOR VVA/GSM TREATMENT

The chronic nature of VVA/GSM indicates that effective treatments should preferably be prescribed at the onset of the symptoms and signs of atrophic changes of the vagina, early before severe pictures of the condition occur, and should be continued over time in order to maintain their benefits (109). The therapeutic approach needs to be personalized and women's preferences have to be taken into account because the level of comfort with a given therapy is strongly influenced by a multitude of individual and socio-environmental factors (110). Apart the embarrassment to discuss an intimate condition, fears of hormones are a major barrier (24), in spite of the very reassuring safety data obtained with local estrogen therapy (LET) (111), the first-line hormonal treatment for VVA/GSM according to guidelines of menopausal scientific societies (53, 112, 113). Various local estrogen treatments are equally effecting in reversing VVA/GSM symptoms, including dyspareunia and other associated sexual dysfunction, alone or even combined with systemic HT. With low-dose LET, systemic estrogen absorption is minimal, and serum estradiol levels remain in the postmenopausal range permitting the use in women with or at high risk for breast cancer, after a discussion of risks and benefits and review with oncologists (76). Local androgens, such as DHEA pessaries and testosterone cream, are new therapeutic options that await for further confirmation (49). Another option approved by Medical Authorities is ospemifene, a third-generation selective estrogen receptor modulator, which is an oral medication for the treatment of VVA associated symptoms (114). It is currently indicated for women, who are not candidates for LET or whenever other treatments, including LET, were not effective to relieve vaginal dryness and dyspareunia (15).

Non-hormonal strategies may be used in women of any age in which hormonal treatments are contraindicated or co-treating women prescribed with systemic/vaginal hormone therapy. The prescription of vaginal moisturizers and lubricants and the maintenance of sexual activity may be helpful in improving vaginal dryness-related symptoms. However, a few clinical trials have been performed to assess the efficacy of such products. Lubricants are short-acting substances (water-, silicone-, or oil-based) which are useful to reduce friction during sexual activity, whereas moisturizers are longer acting than lubricants and may exert a trophic effect (115). Pelvic floor muscle training (PFMT) program in postmenopausal women with urinary incontinence is feasible and improves VVA/GSM symptoms and signs, as well as displays a positive impact on activities of daily living, QoL and sexual function (116). Microablative fractional CO_2 laser, the non-ablative vaginal Erbium YAG laser (VEL) and energy-based devices are increasingly used to alleviate VVA/GSM symptoms with promising results and a good safety profile (117).

VVA/GSM AS A NEGATIVE MARKER OF WOMEN'S AGING: IS THERE ENOUGH EVIDENCE?

Urogenital and sexual longevity is an integral part of healthy aging in postmenopausal women and their partners. The severity of VVA/GSM and the type of prevailing symptoms are mostly influenced by the multitude of clinical phenotypes of postmenopausal women depending on a wide range of biopsychosocial variables which are difficult to estimate in large scale trials. It is known that women loose less years of sexually active life because of poor health than men (118). This data confirm the multidimensional nature of women's sexuality with psychosocial factors (relationship satisfaction, communication with romantic partner, and importance of sex) mattering more than biological aging to sexual satisfaction among midlife and older women (84). That being so, the presence of severe VVA/GSM cannot be considered a negative marker of general health as it had been demonstrated for erectile dysfunction in aging males (119). However, coital sexual activity is associated with an excellent or very good general health also in women, as it is in men (83), and it is certainly influenced by a healthy genital response. Even if it has been difficult to establish a clear link between cardiovascular and metabolic health and women's sexual dysfunctions (120), there is no doubt that several chronic conditions may be associated with poor sexual functioning (121). It is fascinating to speculate on the evidence that vaginal dryness is the only other symptom very sensitive to estrogen deprivation apart hot-flushes (13). Given the clear association of vasomotor symptoms with negative long-term health consequences across aging (15), we cannot exclude that even severe VVA/GSM may represent an early marker of poor general health, a hypothesis that needs further exploration by investigating objective parameters of such chronic condition in relationship with other aspects of women's well-being. Interestingly, baseline characteristics and medical history were tabulated for a VVA cohort identified from two US administrative claims databases (9,080 women aged 40–79 years) and matched controls without VVA. The mean age at baseline was 60.2 years for both but the Deyo-Charlson comorbidity index was significantly higher, with a significantly higher proportion of women in the VVA cohort with a diagnosis of angina, osteoporosis, migraines, insomnia, or anxiety. As expected VVA patients had a significantly higher incidence of each of six genitourinary conditions ("urinary tract infections," "other/unspecified genitourinary symptoms," "other inflammatory diseases of female pelvic organs," "menopausal disorders," "female genital pain and other symptoms," and "other/unspecified female genital disorders") compared to controls (122).

CONCLUSIONS

The management of VVA/GSM is increasingly important in light of the feminilization of aging. Postmenopausal women are becoming aware that preserving urogenital and sexual longevity is a major step in gender equality and healthy living. HCPs should address the issue in daily clinical practice with the aim to prevent the long-term health consequences associated with estrogen deprivation (123). Early recognition of signs and symptoms of VVA/GSM, individual counseling and personalized treatment strategies are key-steps in helping women to maintain QoL.

AUTHOR CONTRIBUTIONS

RN: conception and design. EM, LC, LT, AI, EB, SM, and DB: acquisition, analysis, and interpretation of data. CC and BG: drafting the article. RN and BG: revising for intellectual content. RN, EM, LC, SM, LT, AI, DB, CC, and BG: final approval.

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REVIEW
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Role of Aldosterone and Mineralocorticoid Receptor in Cardiovascular Aging

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The mineralocorticoid receptor (MR) was originally identified as a regulator of blood pressure, able to modulate renal sodium handling in response to its principal ligand aldosterone. MR is expressed in several extra-renal tissues, including the heart, vasculature, and adipose tissue. More recent studies have shown that extra-renal MR plays a relevant role in the control of cardiovascular and metabolic functions and has recently been implicated in the pathophysiology of aging. MR activation promotes vasoconstriction and acts as a potent pro-fibrotic agent in cardiovascular remodeling. Aging is associated with increased arterial stiffness and vascular tone, and modifications of arterial structure and function are responsible for these alterations. MR activation contributes to increase blood pressure with aging by regulating myogenic tone, vasoconstriction, and vascular oxidative stress. Importantly, aging represents an important contributor to the increased prevalence of cardiometabolic syndrome. In the elderly, dysregulation of MR signaling is associated with hypertension, obesity, and diabetes, representing an important cause of increased cardiovascular risk. Clinical use of MR antagonists is limited by the adverse effects induced by MR blockade in the kidney, raising the risk of hyperkalaemia in older patients with reduced renal function. Therefore, there is an unmet need for the enhanced understanding of the role of MR in aging and for development of novel specific MR antagonists in the context of cardiovascular rehabilitation in the elderly, in order to reduce relevant side effects.

Keywords: endothelial dysfunction, mineralocorticoid receptor, vascular stiffness, RAAS, oxidative stress

INTRODUCTION

The mineralocorticoid receptor (MR) is essential for blood pressure regulation and electrolyte and fluid homeostasis (1). MR activation by aldosterone evolved in response to dramatic changes in salt stress which occurred during the transition from aquatic to terrestrial life. Indeed, aldosterone first appeared in tetrapods (2) suggesting that the aldosterone-MR system was necessary to maintain ion balance during the transition from salt water to land. In mammals, the kidney maintains osmolarity and extracellular sodium concentration, as well as plasma volume and blood pressure (3). Aldosterone is produced by the adrenal glands and represents the most potent sodium-retaining hormone in mammals (4). Aldosterone secretion is stimulated under specific conditions, such as the increase in extracellular K^+ ion concentrations or renin-angiotensin-aldosterone system (RAAS) activation in response to decreased vascular volume (5, 6). In addition to its well-established role in the kidney, MR is expressed in many non-epithelial tissues [i.e., adipose tissue (AT), heart, endothelial cells, vascular smooth muscle cells, brain, etc.]. In this context, abnormal MR activation contributes to relevant cardiovascular alterations by multiple mechanisms including enhanced oxidative stress, inflammation, fibrosis, vascular tone, and endothelial dysfunction (7). Importantly, MR displays a similar affinity for aldosterone and the physiological glucocorticoids (cortisol and corticosterone) (8). In epithelial tissues, as well as in endothelial cells (9) and smooth muscle cells (10), the enzyme 11b-hydroxysteroid dehydrogenase type 2 (11HSD2) is able to convert endogenous glucocorticoids to inactive metabolites (11), promoting MR activation by aldosterone. In non-epithelial tissues, where expression of 11HSD2 is virtually absent or extremely low, such as brain, cardiomyocytes, and adipose tissue, glucocorticoids represent the major ligand of the MR (12).

Aging is a universal and independent risk factor for cardiovascular diseases (CVD) including hypertension, coronary artery disease, congestive heart failure and stroke (13, 14). According to a report from the American Heart Association (15), the incidence and prevalence of CVD significantly increases with age, and about two-thirds of CVD deaths occur in people aged 75 and older. To date, the influence of aging on aldosterone secretion and function in humans is not well-characterized, and the specific role of MR activation in vascular aging still awaits demonstration. In animal models, MR contributes to rising blood pressure with aging by regulating myogenic tone, vasoconstriction, and vascular oxidative stress (16). Both oxidative stress (17) and inflammation (18) are key factors in the pathophysiology of age-related cardiovascular disease in humans. Telomeres length in white blood cells can be considered as a biomarker of oxidative stress and inflammation as their progressive attrition, due to cell replication, is increased by oxidative stress, and inflammation determines an increase in white blood cells turnover rate. White blood cells telomeres are shorter in CVD patients. Aldosterone is known to accelerate cardiovascular aging through processes that generate reactive oxygen species in several tissues as well as in white blood cells (19–22) and an inverse relationship between circulating aldosterone and white blood cells telomeres length has been documented in normotensive aged matched men (23).

Several recent studies showed that MR expression is increased in vascular smooth muscle cells of aged animals (24, 25). Molecular mechanisms have also been uncovered by which rising vascular smooth muscle cell MR contributes to increased vasoconstriction with aging (26). Moreover, recent histopathologic findings have clarified profound alterations of the zona glomerulosa in adrenal glands with aging, which together with the increased vascular MR expression, may provide a further explanation for enhanced cardiovascular risk in the elderly (27, 28).

In this review, we will focus on the age-related alterations of MR signaling and aldosterone secretion and will discuss their specific role in determining increased cardiovascular risk in the elderly. Finally, we will address the potential relevance of MR pharmacological antagonism in the elderly, in order to increase arterial compliance and prevent cardiovascular aging and the associated morbidity and mortality.

RAAS ALTERATIONS WITH AGING

Several studies have shown that older healthy individuals display a reduction in renin-angiotensin-aldosterone system (RAAS) activity, with decreased plasma renin activity and lower levels of plasma renin and aldosterone under basal conditions (hyporeninaemic hypoaldosteronism) (29–33). The decline in plasma renin with age has been attributed to the effect of age-associated nephrosclerosis (34). Human studies with small sample sizes suggested that older individuals secrete less aldosterone than younger ones (35), resulting in a greater risk for hyperkalemia in older individuals (36), especially when coupled with the age-associated decline in glomerular filtration rate (GFR). Accordingly, renin synthesis and release gradually decrease in aging rats, resulting in lower levels of plasma renin (37). Moreover, older subjects also show an impaired ability to trigger adequate responses to RAAS stimuli, such as orthostatic hypotension, potassium infusion or sodium depletion (29, 38).

These age-related RAAS alterations have been attributed to different mechanisms occurring with aging, namely: (i) glomerulosclerosis and reduction in functional nephrons (39–41); (ii) impaired function of juxtaglomerular apparatus (e.g., reduced sympathetic stimulation of the juxtaglomerular apparatus) (39); (iii) reduced renal production of kallikrein (a serine protease contributing to the synthesis of active renin); and (iv) reduced angiotensinogen synthesis by the liver (39, 42).

Importantly, age-related changes in RAAS activity lead older individuals to reduced ability to reabsorb sodium and reduced renal tubular potassium excretion, resulting in higher risk for volume depletion, hyponatremia and/or hyperkalemia (36). Of note, the risk for hyperkalemia is further enhanced under specific conditions, such as metabolic acidosis, reduction in GFR, or use of drugs inhibiting renal tubular potassium excretion [i.e., angiotensin converting enzyme (ACE) inhibitors, angiotensin II (Ang II) type 1 (AT1) receptor antagonists, MR antagonists, non-steroidal anti-inflammatory drugs (43)].

Recent reports clarified the histopathological changes occurring in adrenal glomerulosa cells with aging (27). The development of specific antibodies against aldosterone synthase (CYP11B2—the enzyme required for the final step of aldosterone production) recently allowed the detection of non-neoplastic foci of CYP11B2-expressing cells in the adrenal, referred to as aldosterone-producing cell clusters (APCC), which are commonly observed in normal human adrenals. Interestingly, recent studies revealed that the classic continuous CYP11B2 expression pattern within adrenal zona glomerulosa is gradually lost with aging, whereas accumulation of APCC in adrenal glands is frequently observed with advancing age. A direct evidence that APCC autonomously secrete aldosterone still awaits demonstration; however, aging is characterized by the transition from a physiological aldosterone regulation to a pattern of renin-independent aldosterone secretion, which could be sustained by increased number in APCC (28), and may account, at least in part, for the increased cardiovascular risk observed in the elderly (27).

Finally, it has been also shown that aging is associated with a decline in 11HSD2 activity, which results in renin suppression and cortisol-mediated MR activation (44), thus providing another potential mechanism for enhanced MR activation with aging.

Together, previous studies from both humans and animals provide evidence of altered RAAS activity and secretion with aging, which play a pivotal role in pathogenesis of CVD.

ROLE OF THE MINERALOCORTICOID RECEPTOR IN VASCULAR DYSFUNCTION WITH AGING

Aging is associated with structural, mechanical and functional alterations in the vasculature that are characterized by augmented vasoconstriction, reduced elasticity and distensibility, vascular stiffening, and impaired endothelial function (14, 45). These aging-related vascular changes contribute to cardiovascular disease and may be reversible; therefore, elucidating the mechanisms driving vascular aging has substantial potential to identify new therapeutic targets to prevent or reverse vascular aging, thereby attenuating the high CVD burden in the rapidly growing elderly population.

In addition to the traditional role of renal MR in regulating blood pressure by promoting sodium retention in the kidney (46), accumulated data in the past two decades indicate that MR is also expressed in the vasculature, including the smooth muscle cells, that contribute to vascular structure and vasoconstriction, and the endothelial cells, that contribute to barrier function and inflammation and thrombosis when injured (9, 10, 26). Substantial evidence support that MR in vascular cells contributes to CVD [reviewed elsewhere (47, 48)]. Animal studies have demonstrated that treatment with MR antagonists ameliorates vascular remodeling and dysfunction in the setting of CVD risk factors, including aging, western diet-induced obesity and hypertension, without significantly altering blood pressure (49–52), suggesting direct effects of MR antagonism on the vasculature. In clinical studies, MR antagonist treatment reduced vascular stiffness in elderly patients particularly with hypertension (53, 54).

MR expression increases in the vasculature with aging. Krug et al. found that MR gene expression is higher in aortas from aged rat (30 months of age) than in aortas from adult rat (8 months of age), and that MR protein expression was increased with aging in isolated rat aortic smooth muscle cells (24). More recent studies have similarly shown increased MR gene expression in mouse mesenteric resistance arteries with aging (25). To investigate the specific role for vascular smooth muscle cells MR in age-related mechanical and functional changes in the vasculature with aging, mice with smooth muscle cell-specific deficiency of MR (SMC-MR-KO) have been generated (26). Using these mice, McCurley et al. found that the moderate rise in blood pressure with aging in mice is prevented in SMC-MR-KO mice, without defects in renal function. Compared to aged MR-intact mice, 12 month-old SMC-MR-KO mice also showed decreased myogenic tone, vasoconstriction, and voltage-gated calcium channel expression, and decreased oxidative stress both at baseline and in response to Ang II (26). These findings indicate a direct contribution of smooth muscle cells-MR to increased vasoconstriction, vessel tone, and oxidative stress in aging vessels, which may contribute to the inexorable rise in blood pressure with aging. Further exploration of the mechanism by unbiased global miRNA expression profiling in mouse aortas, identified microRNA (miR)-155 as the most down-regulated miRNA in the aging vasculature. Interestingly, such down-regulation was prevented in SMC-MR-KO mice (25). DuPont et al. further demonstrated that MR transcriptionally represses the miR-155 host gene promoter. Thus, the increase in vascular MR expression with aging was associated with repression of miR-155 and increased expression of miR-155 target genes including the L-type calcium channel *(LTCC)* subunit Cav1.2 and the Ang II type 1 receptor (*Agtr1*), which are known to contribute to vasoconstriction and vascular oxidative stress with aging. These aging effects were prevented in SMC-MR-KO mice further supporting this as a mechanism by which smooth muscle cells-MR contributes to increased vasoconstriction, vessel tone and oxidative stress during aging (25).

Smooth muscle cells-MR was also recently found to contribute to vascular structural changes with aging that determine vascular stiffening (16), a prominent consequence of aging in humans that correlates with risk of cardiovascular events (14, 45, 55). Although multiple CVD risk factors accelerate vascular stiffening, aging itself is associated with vascular stiffening that can occur independently and may even contribute to the development of other risk factors including hypertension (55–57). An important cause of vascular stiffness is excessive vascular fibrosis and reduced elasticity (45). Comparison of vascular stiffness with aging in MR-intact mice revealed increased aortic stiffness in 12 month- and 18 month-old mice compared to 3 month-old mice, along with increased fibrosis in aorta, carotid arteries and renal arterioles. These aging-associated increases in vascular stiffness and fibrosis were mitigated in SMC-MR-KO mice (16). Gene expression profiling in aortas revealed that MR deletion in smooth muscle cells induces a distinct anti-fibrotic gene profile in the aging vasculature, including downregulation of well-characterized pro-fibrotic genes such as connective tissue growth factor (CTGF), matrix metalloprotease-2 (MMP2), and bone morphogenetic protein-4 (BMP4) (16), that contribute to vascular fibrosis (14, 45). These findings indicate a role for smooth muscle cell-MR in vascular aging as a transcriptional regulator that activates pro-fibrotic genes with aging, consistent with prior studies showing that aldosterone activates pro-fibrotic genes in mouse vessels (58) and in human coronary artery smooth muscle cells (10). Moreover, long-term treatment of aged mice with MR antagonist prevented the progression of vascular stiffening, reduced vascular fibrosis and induced a similar anti-fibrotic gene signature as smooth muscle cell-MR gene deletion (16). A small cohort study in humans also showed that MR antagonism treatment for 1 month reduced fibrotic biomarkers in the serum from elderly patients compared to placebo treatment (16). Altogether, the available preclinical data reveal that MR expression in smooth muscle cells of the vasculature increases with aging and induces structural, mechanical, and functional changes in vessels that contribute to vascular stiffness and to rising blood pressure with age (Figure 1). Mechanistically, smooth muscle cell-MR contributes to functional and structural alterations of vessels with aging through the role of MR as a transcriptional regulator of genes associated with vascular tone, oxidative stress and fibrosis. Although larger and longer clinical studies in elderly humans are warranted, these findings support the potential benefits of MR antagonism to treat vascular aging and associated morbidity with aging.

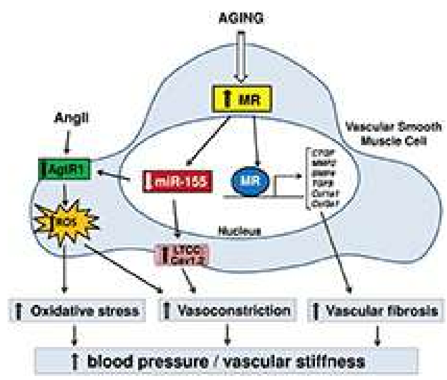

FIGURE 1. Diagram represents signaling for the contribution of mineralocorticoid receptor (MR) in smooth muscle cell (SMC) to vascular aging. Rises in SMC-MR expression with aging suppress miR-155, leading to the up-regulation of angiotensin type 1 receptors (AgtR1) and L-type calcium channels (LTCC), resulting in increased calcium influx and reactive oxygen species (ROS) production. This signaling causes enhanced vasoconstriction and oxidative stress. Also, increased MR in SMC with aging contributes to transcriptional activation of pro-fibrotic genes, leading to increased vascular fibrosis. These structural and functional changes with aging via MR in SMC result in hypertension and vascular stiffening. CTGF, connective tissue growth factor; MMP2, matrix metalloproteinase-2; BMP4, bone morphogenetic protein-4; TGFβ, transforming growth factor beta; Col1a1, collagen type-1 alpha-1; Col3a1, collagen type-3 alpha-1; Cav1.2, calcium channel; voltage-dependent; L type, alpha 1C subunit.

To our knowledge, the specific role of MR in other vascular cells, such as endothelial cells, myeloid cells, fibroblasts, or perivascular adipose cells, has not been directly investigated in the setting of aging. However, studies have demonstrated that endothelial cell-specific MR deficiency or MR antagonists treatment in mice prevents hormone- or diet-induced increases in endothelial cell stiffness, oxidative stress, leukocyte adhesion and the associated decrease in nitric oxide (NO) production (59, 60), which are prominent features of age-related vascular dysfunction (48). In addition, although smooth muscle cell MR does not contribute to atherosclerosis (61), endothelial cell MR has recently been implicated in vascular inflammation in mouse models of atherosclerosis, specifically in males (62). Prior studies have also implicated MR expressed by myeloid cells in atherosclerosis, in vascular inflammation, fibrosis and remodeling as well as T-cell MR in hypertension (63–65). Thus, MR in other cells contributes to important vascular phenotypes that are known to be associated with vascular aging, supporting the need for future studies to investigate directly the roles for non-smooth muscle cells MR in vascular aging.

ROLE OF MINERALOCORTICOID RECEPTOR IN MYOCARDIAL DYSFUNCTION WITH AGING

The aging heart is characterized by various functional and structural changes, partially resembling some of the features observed in animal models of increased MR activation (66), such as inflammation, oxidative stress, collagen accumulation and fibrotic remodeling (66–68). A growing body of evidence has suggested an important contribution of aldosterone and MR activation to cardiac remodeling and heart failure (69, 70). MR expression was first detected in cardiomyocytes and endothelial cells of atria and ventricles almost 30 years ago (71). In the myocardium, MR is also expressed in cell types other than cardiomyocytes, including coronary vasculature and macrophages (71, 72). Interestingly, experimental studies have shown that mice with cardiomyocyte-specific overexpression of MR display oxidative stress-mediated coronary endothelial dysfunction and increased expression of pro-fibrotic markers (e.g., CTGF) (67, 69, 73). Wilson et al. demonstrated that rats exposed to mineralocorticoids excess undergo a series of inflammatory and oxidative stress responses before the onset of myocardial hypertrophy or fibrosis (74). A recent publication by Kim et al. indicates that smooth muscle cell-MR deletion attenuates aging-associated increases in cardiac stiffness. The increase in cardiac systolic stiffness with aging correlated with the degree of aortic stiffness, suggesting that cardiac benefits of smooth muscle cell MR deletion in mice may be secondary to the prevention of vascular stiffening (16).

Macrophage MR has been also found to play a key role in mediating cardiac tissue remodeling, stimulating the pro-inflammatory macrophage M1-like phenotype (known as “classically activated” macrophages) and regulating the transcription of different inflammatory and pro-fibrotic markers, such as tumor necrosis factor α (TNFα) and transforming growth factor β1 (TGF-β1) (68, 75). MR is also expressed on T lymphocytes and its overactivation upregulates CD8+ cytotoxic T cells and T helper 17 (Th17) cells infiltrating in the heart. Other studies showed that MR antagonism decreases Th17 polarization and induces the T regulatory cells phenotype (76, 77). Interestingly, pharmacological MR antagonism decreased the accumulation and activation of CD4+ and CD8+ T cells in the murine heart and T cells specific MR-knockout mice displayed reduced cardiac hypertrophy, fibrosis, and dysfunction (78). Moreover, the MR selective antagonist eplerenone improved the adverse cardiac effects of aging in spontaneously hypertensive rats, reducing myocardial fibrosis and improving left ventricular diastolic function and coronary hemodynamics (79).

MR activation can also affect myocardial electrical function, potentially causing lethal cardiac arrhythmias associated with heart failure (70, 80). Gómez et al. demonstrated that the overstimulation of cardiac MR pathway leads to increased ryanodine receptor activity and long-lasting and broader spontaneous calcium sparks, which potentially predispose to arrhythmias (81). Another study has shown that transgenic mice with cardiac-selective overexpression of human MR exhibit a high rate of death due to ion channel remodeling (reduced outward K^+ transient current, increased Ca^{2+} influx), which results in prolonged ventricular repolarization and fatal ventricular arrhythmias in absence of structural cardiac defects. Importantly, administration of spironolactone in pregnant mice was able to prevent embryonic and postnatal death in the offspring, suggesting that offspring lethality was highly related to MR overexpression and activation (82).

Atrial fibrillation is the most frequent cardiac arrythmias in the elderly population (83). Interestingly, Tsai et al. found that atrial MR expression is significantly higher in patients with atrial fibrillation compared with individuals with normal sinus rhythm. In the same study, aldosterone increased the expression of α-1G and−1H subunits of the T-type calcium channel in cultured murine HL-1 atrial myocytes, leading to increased T-type calcium current and calcium overload, which was attenuated by the mineralocorticoid antagonist spironolactone (84). Accordingly, although there is no evidence showing a direct role of MR dysfunction in aging causing atrial fibrillation, we can speculate that increased MR signaling in heart tissue, due to aging, could represent a causal link between aging and atrial fibrillation. Further studies are needed to directly explore this possibility.

In summary, accumulating data demonstrate that MR contributes to aging-associated myocardial dysfunction with cell type-dependent mechanisms revealed by animal studies, thus supporting the potential benefits of MR antagonism to treat cardiac dysfunction, especially in elderly population.

ROLE OF MINERALOCORTICOID RECEPTOR IN ENDOTHELIAL DYSFUNCTION AND INFLAMMATION

WITH AGING

Very little is known about the role of aldosterone and MR activation in the vasculature in the context of healthy human aging. Healthy endothelial cells secrete vasodilator mediators which activate signaling pathways inducing smooth muscle cells to relax and leading to vasodilation (85). Nitric oxide (NO) is produced by healthy ECs after activation of endothelial nitric oxide synthase (eNOS). NO represents a major mediator of endothelial-dependent vasorelaxation (86, 87). In patients with cardiovascular risk factors, such as hypertension, obesity and diabetes, extensive data demonstrate that MR activation contributes to endothelial dysfunction, through impairment of vasodilation induced by the endothelium (22, 85, 88–91). In human coronary endothelial cells, MR regulates several genes involved in inflammation and oxidative stress (9, 10). It is known that MR activation in endothelial cells contributes to cardiac inflammation and remodeling by promoting the expression of vascular cell adhesion molecule 1 (VCAM1), as shown in animal models of hypertension (92). Moreover, aldosterone-mediated endothelial MR activation leads to the overexpression of the intracellular adhesion molecule-1 (ICAM-1), thereby enhancing leukocyte adhesion to coronary artery endothelial cells (9, 93). *In vivo*, MR in the endothelium contributes to ICAM-1 and E-selectin expression thereby contributing to leukocyte slow rolling and adhesion to the vasculature, a critical step in the process of inflammation (62).

Reactive oxygen species have also been suggested to mediate the detrimental effects of aldosterone in the vasculature through MR activation (94, 95). Arterial superoxide levels increase with aging, in part because of the excessive activity of NADPH oxidase. Increased oxidative stress leads to the inactivation of nitric oxide (96) and consequent arterial stiffness (97). Several studies showed that MR activates NADPH oxidase-dependent superoxide production (22, 90) and MR blockade decreases NADPH oxidase activity, reduces superoxide formation, and improves nitric oxide bioavailability (98). Importantly, the sensitivity of the MR to aldosterone is enhanced in arteries from aged and/or hypertensive humans (99). In animal models with enhanced cardiovascular risk, endothelial dysfunction is driven by aldosterone activation of endothelial cell-MR. Spironolactone significantly improved endothelial function in middle cerebral artery in a spontaneously hypertensive rat model (100). Moreover, pharmacological MR inhibition or selective deletion of MR in endothelial cells prevented impaired vasodilation in a model of diet-induced obesity (101) specifically in females (102). Finally, selective endothelial cell MR deletion in mice improved endothelial dysfunction upon a challenge of Ang II induced hypertension (103).

Only few clinical studies evaluated the effects of MR antagonists on arterial stiffness in hypertensive patients. In two different studies, eplerenone showed higher efficacy in reducing arterial stiffness than atenolol and a thiazide type diuretic (53, 104). On the other hand, a study comparing eplerenone and amlodipine showed that the aortic pulse wave velocity decreased similarly in both groups (105). Interestingly, in a randomized study conducted by Hwang et al. on healthy older adults free from overt cardiovascular disease, pharmacological inhibition of MR did not decrease oxidative stress nor lead to improved arterial stiffness and wave reflections. These findings suggest that MR may not substantially contribute to oxidative stress in healthy human aging in the absence of additional risk factors (106). The same authors also showed that acute inhibition of MR in healthy aged adults led to impairments in vascular endothelial function, suggesting that the MR may induce beneficial physiological actions in regulating eNOS activity and flow-mediated endothelium-dependent dilation in healthy aging (107). Vascular smooth muscle responsiveness to exogenous nitric oxide was not influenced by acute MR antagonism in this population. Similarly, acute MR antagonism did not affect systemic blood pressure or circulating and endothelial cell markers of oxidative stress and inflammation (107). Other studies demonstrated that MR deletion in endothelial cells does not inhibit endothelium-dependent relaxation in healthy aorta (101), mesentery and coronary arteries (103). Conversely, in subjects with CVD risk factors, endothelial dysfunction seems to be dependent on MR activation. In this regard, studies conducted in animal models suggest that the specific role of MR activation in endothelial function depends on endothelial health and integrity (102, 108). Thus, it can be speculated that MR activation determines the induction of a vasodilatory response in healthy endothelium, and a vasoconstriction response (potentially mediated by smooth muscle cell–MR) when the endothelium is stressed or damaged.

Aging is associated with a progressive worsening of several physiological processes, leading to an increased risk of diseases, particularly at cardiovascular level (13, 14). Aging causes a pro-inflammatory state, remodeling of the vasculature, endothelial dysfunction and excessive production of reactive oxygen species (13, 14, 96, 109), mainly by increased expression and activity of NAD(P)H oxidase, which is not efficiently countered by antioxidant enzymes (110, 111). In the elderly, oxidative stress represents the most important cause of epigenetic modification (112) of the genes encoding for the antioxidant enzyme superoxide dismutase (113). In addition, the increased endoplasmic reticulum stress and proteasome activity elicits the process of unfolded protein response in vascular smooth muscle cells, monocytes, and endothelial cells (114). In this particular context of unhealthy aging, characterized by vascular damage, endothelial cell-MR activation can amplify cardiovascular adverse outcomes, exacerbating vascular stiffness through the induction of augmented reactive oxygen species production, collagen deposition, and vascular inflammation (9, 94, 95), resulting in altered vasodilation, endothelial dysfunction, and atherosclerosis (Figure 2).

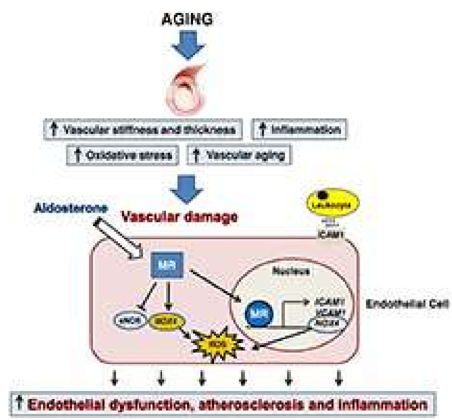

FIGURE 2. Aging is associated with vascular damage characterized by inflammation, vascular thickening, arterial stiffness and overproduction of reactive oxygen species (ROS). In this setting, mineralocorticoid receptor (MR) activation in endothelial cells can contribute to amplify cardiovascular adverse outcomes, exacerbating vascular stiffness through the induction of augmented ROS production, vascular inflammation and collagen deposition, finally leading to endothelial dysfunction and atherosclerosis. eNOS, endothelial nitric oxide synthase; NOX4, NADPH Oxidase 4; ICAM1, Intercellular Adhesion Molecule 1; VCAM1, vascular cell adhesion molecule 1.

In summary, a large body of evidence indicate that endothelial cell-MR is implicated in the pathological outcomes of cardiovascular risk factors, which are also highly associated with aging. Future studies are needed to determine if endothelial cell-MR plays a direct role in cardiovascular aging in animal models and humans.

MR ANTAGONISTS IN THE ELDERLY: CLINICAL STUDIES

MR antagonists are largely used for the treatment of resistant hypertension and hearth failure (HF) (115), which represent highly prevalent diseases among older individuals (116, 117). In this context, several clinical trials demonstrated that cardiovascular morbidity and mortality are significantly reduced from the use of MR antagonists in moderate to severe heart failure with reduced ejection fraction (HFrEF) (118–120). In the double-blind Randomized Aldactone Evaluation Study (RALES), 1,663 patients with severe HFrEF and an average age of 65 years were randomly assigned to receive the MR antagonist spironolactone or placebo. After a mean follow-up period of 24 months, individuals from the spironolactone group showed a significant improvement in the symptoms of heart failure and a significant reduction in mortality, the latter attributed to the lower risk of death from cardiac causes (118). Thereafter, the Eplerenone Post-AMI Heart Failure Efficacy and Survival Study (EPHESUS) investigated the effects of the selective MR antagonist eplerenone on morbidity and mortality among 6,642 patients with an average age of 64 years and HFrEF following an acute myocardial infarction. After a mean follow-up period of 16 months, eplerenone significantly reduced the risk of death and hospitalization from cardiovascular causes and from any cause, as well as the rate of sudden death from cardiac causes (120). In contrast with these findings, the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist trial (TOPCAT) found that spironolactone did not significantly reduce the rates of the primary composite outcome of death from cardiovascular causes, cardiac arrest, or hospitalization for heart failure in patients with heart failure with preserved ejection fraction (HFpEF) and a median age of 68.7 years (121). However, a *post-hoc* analysis has shown that spironolactone significantly reduced the TOPCAT primary outcome in patients with HFpEF from the Americas, suggesting that differences in demographic characteristics among recruited individuals may have represented a relevant bias of the study (122). On the other hand, a meta-analysis of seven randomized controlled trials evaluating the impact of MR antagonists on cardiovascular mortality and morbidity outcomes in patients with heart failure and/or left ventricular systolic dysfunction aged ≥65 years, did not confirm significant improvement in clinical outcomes among patients with HFpEF. However, the same study showed that MR antagonism improves clinical outcomes in selected cohorts of older patients with HFrEF (123). Another sub-analysis, which included 1,767 of the TOPCAT patients and was equally comprised of men and women, demonstrated that women with HFpEF had a significant reduction in cardiovascular and all-cause mortality with spironolactone, while men did not (124).

Interestingly, MR antagonists were also found to exert clinical benefit in patients with atrial fibrillation. In particular, a clinical trial on 164 patients aged

≥66 years with recurring atrial fibrillation showed that spironolactone, administered with β-blockers, was able to significantly prevent arrhythmic events, compared to spironolactone untreated patients (125). Recently, a retrospective cohort study of the contemporary ORBIT-AF (Outcomes Registry for Better Informed Treatment of Atrial Fibrillation) registry showed that the use of MR antagonists was not associated with reduced atrial fibrillation, but showed a trend toward lower risk of stroke, transient ischemic attack, or systemic embolism (126). However, the hypothesis that MR antagonists therapy may reduce residual stroke risk in patients with atrial fibrillation awaits demonstration in randomized clinical trials.

The recent 2018 ESC/ESH guidelines for the management of arterial hypertension now recommend that systolic blood pressure should be targeted to a range of 130–139 mmHg in older (>65 years) and very old (>80 years) patients (127). Importantly, recommended treatment of resistant hypertension considers the addition of low-dose spironolactone (up to 50 mg/day) to existing therapy also in the elderly population, where loop diuretics and alpha-blockers should be avoided due to their association with falls (128), extending the possibility of pharmacological MR antagonism in the aging hypertensive population.

In light of the significant cardiovascular benefits of MR antagonism in the aging population, their use in clinical setting is limited by the adverse effects induced by MR blockade on the kidney, such as hyperkalemia, particularly in older patients with reduced renal function and by their anti-androgenic properties (particularly exhibited by spironolactone) which can induce gynecomastia and erectile dysfunction in men (129, 130). Therefore, the current use of MR antagonists is restricted to patients with an estimated glomerular filtration rate >45 mL/min and a plasma potassium concentration of <4.5 mmol/L, in order to avoid the risk of hyperkalaemia (127). For such reasons, there is an unmet need for the development of more selective MR antagonist for heart and vasculature, in order to minimize the relevant side effects on non-cardiac tissues.

CONCLUDING REMARKS

It is now clear that altered MR function is involved in the pathophysiology of endothelial dysfunction, atherosclerosis, oxidative stress, and cardiac remodeling. Altogether, these conditions are highly prevalent in the aging population and are deeply involved in the development of ischemic events and heart failure, common causes of morbidity and death in the elderly. Several recent studies demonstrated that aging is associated with important alterations in the aldosterone-MR system with changes in aldosterone production by the aging adrenal and increased MR responsiveness by the aging cardiovascular system. In accordance, clinical trials revealed the efficacy of MR antagonism in improving cardiovascular morbidity and decreasing mortality. The mechanisms involved in these cardiovascular benefits are complex and well beyond their well-known blood pressure lowering effects. It is now clear that systemic pharmacological antagonism produces direct effects in the vasculature and heart. However, MR pharmacological blockade in clinical practice has been limited by the risk of important adverse effects, such as hyperkalemia and renal dysfunction worsening, which is particularly frequent in aged individuals. Recently, a novel class of non-steroidal MR antagonist has been developed (131). Finerenone belongs to this group of molecules and its MR selectivity and affinity are higher compared to spironolactone and eplerenone. Due to these differences, finerenone may potentially reduce risk of both hyperkalaemia and renal impairment and, if so, may be safer to use in patients with heart failure affected by chronic renal dysfunction (132). Specifically, five phase II clinical trials demonstrated that finerenone is safe in patients with heart failure and concomitant chronic renal impairment and/or diabetes mellitus, and neither hyperkalemia nor reductions in kidney function were limiting factors to its use in over two thousand patients (133). Such favorable side effects profile is reached in the presence of similar clinical efficacy compared to other MR antagonists. Importantly, the addition of finerenone in patients with diabetic nephropathy resulted in improvement in the urinary albumin-creatinine ratio (134). ARTS-HF was the first clinical trial to compare finerenone with eplerenone, in patients with worsening HFrEF and chronic kidney disease and/or diabetes mellitus, with a mean age of 71.5 years. In such vulnerable population, finerenone reduced levels of NT-proBNP to a similar extent to that of eplerenone, but showed less changes in serum potassium from baseline to the end of the study in comparison to eplerenone (135). Importantly, finerenone at a dose of 10–20 mg demonstrated a nominally improved outcome of a composite clinical endpoint of death from any cause, CV hospitalizations, or emergency presentation for worsening heart failure (hazard ratio, HR: 0.56 [95% CI: 0.35–0.90]) compared to eplerenone in ARTS-HF. Moreover, preclinical studies showed that finerenone was able to potently block cardiac fibrosis and macrophages infiltration in a mouse model of isoproterenol-induced cardiac fibrosis, whereas eplerenone did not show significant effects (136). Nevertheless, phase III clinical trials will be crucial to further investigate the efficiency and safety of novel MR antagonists in the aging population, and studies on different subgroups of elderly people will help to identify new strategies to prevent cardiovascular aging, and to reduce the risk of end-organ damage related to MR activation (137).

AUTHOR CONTRIBUTIONS

SG and SK conceived and wrote the manuscript. MI wrote, in part, and revised the manuscript. CM prepared the figures and revised the manuscript. SL, AF, and IJ revised the manuscript. MC conceived, wrote, in part, and revised the manuscript.

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