

MECHANISMS IN ENDOCRINOLOGY

Aging and anti-aging: a Combo-Endocrinology overview

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Abstract

Aging and its underlying pathophysiological background has always attracted the attention of the scientific society. Defined as the gradual, time-dependent, heterogeneous decline of physiological functions, aging is orchestrated by a plethora of molecular mechanisms, which vividly interact to alter body homeostasis. The ability of an organism to adjust to these alterations, in conjunction with the dynamic effect of various environmental stimuli across lifespan, promotes longevity, frailty or disease. Endocrine function undergoes major changes during aging, as well. Specifically, alterations in hormonal networks and concomitant hormonal deficits/excess, augmented by poor sensitivity of tissues to their action, take place. As hypothalamic–pituitary unit is the central regulator of crucial body functions, these alterations can be translated in significant clinical sequelae that can impair the quality of life and promote frailty and disease. Delineating the hormonal signaling alterations that occur across lifespan and exploring possible remedial interventions could possibly help us improve the quality of life of the elderly and promote longevity.

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Invited Author's profile

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Introduction

Aging and the quest for the ‘fountain of youth’ have intrigued the interest and the curiosity of scientific society throughout the history of mankind. Aging is defined as the gradual, time-dependent decline or loss of physiological functions, affecting all living beings. It is a complex and heterogeneous process, as its rate varies considerably between diverse species, between organisms of the same species, even between cells and tissues of the same organism (1).

At a mechanistic level, multiple molecular mechanisms have been associated with the pathophysiology of aging. Incessant accumulation of DNA and cellular damage contribute to the loss of structural and functional homeostasis of cells, triggering ultimately senescence (2). Specifically, in an illuminating review by Lopez-Otin *et al.* nine cellular and molecular hallmarks of aging have been identified. Genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, altered intracellular signaling, stem cell exhaustion and the complex interplay between all the above-mentioned mechanisms lie in the pathophysiological core of aging (3). The ability of an organism to adjust to these alterations, in conjunction with the dynamic effect of various environmental stimuli across lifespan, promotes longevity, frailty or disease.

Hypothalamic–pituitary unit is the central regulator of crucial body functions, including nutrition, growth, metabolism and reproduction. During aging, alterations in hormonal networks and concomitant hormonal deficits/excess, augmented by poor sensitivity of tissues to their action, take place. These alterations are translated clinically to the reduction of skeletal muscle, increased adiposity, bone loss, insulin signaling dysfunction, impaired immune function and, more importantly, decreased quality of life (4).

This review focuses on unraveling the pathophysiological mechanisms, underlying the aging of endocrine system, and delineating the hormonal signaling alterations that occur across lifespan. Additionally, we will clarify how these endocrine changes are translated to frailty and disease and evaluate if and how remedial interventions can improve the quality of life of the elderly and promote longevity.

Aging of endocrine system and oxidative stress

A plethora of pathophysiological theories has been postulated so far to understand the aging process deeper.

Every theory of them proposes a specific pathogenetic mechanism that leads humans grow old, providing useful and important insights for the understanding of physiological changes occurring with aging. Recently, the search for a single cause of aging has been replaced by the view of aging as an extremely complex and multifactorial process. In fact, it is very likely that several processes are closely intertwined and operate at different levels of functional organization to promote aging (5).

One of the first and the most widely accepted theories of aging is the free radical theory of aging, proposed by Harman D approximately sixty years ago (6). Oxidants, including reactive-oxygen species (ROS) and reactive-nitrogen species (RNS), are normal by-products of aerobic metabolism, generated mainly in mitochondria, due to leakage of electrons from electron transport chain (7). Overproduction of oxidants during vital processes of human body that cannot be overpowered by the anti-oxidant defense systems causes irreversible damage of proteins, lipids and DNA and leads ultimately to disrupted cell function (8). Indeed, according to the free radical theory of aging, continuous, unrepaired oxidative damage of macromolecules throughout the lifespan is considered as the molecular basis of aging.

Regarding endocrine system, oxidative stress has also been implicated in the aging of endocrine glands and their concomitant dysfunction. Specifically, according to the revised ‘nitric oxide theory of aging’, free radicals overproduction in central nervous system, including hypothalamic–pituitary axis, might be responsible for the aging of these structures (9). Kondo *et al.* have described an age-dependent accumulation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (10), a powerful oxidant compound, in the human pituitary, while in a study by Nessi *et al.* increased number of apoptotic thyrotroph and somatotroph pituitary cells were observed during aging (11). Additionally, levels of elongation factor 2, an essential factor for protein synthesis, which can be disrupted by lipid peroxidation caused by ROS, were found to be decreased in hypothalamus and pituitary cells of rats during aging (12). Finally, melatonin, which is considered a powerful anti-oxidant compound and scavenger of ROS, decreases and displays impaired diurnal rhythms in elderly (13).

Apart from central nervous system, oxidative stress is involved in the aging process of other endocrine glands, as well. For example, oxidative stress is involved in the pathophysiology of thyroid autoimmune diseases, which

increase during aging, through direct effects on immune system (14). What is more, selenium depletion in aged population makes thyroid more vulnerable to oxidative stress (15). Regarding pancreatic β cells, oxidative stress can significantly compromise their function, as pancreatic β cells are innately more sensitive to oxidative stress, since they are lower in anti-oxidant enzymes levels (16). However, simultaneously, generation of ROS is innately coupled to the glycolytic and respiratory metabolism in β cells. To date, many important signal transduction molecules or processes that potentially regulate glucose-stimulated insulin secretion in β cells have been recognized as downstream targets of superoxide, including voltage-gated K^+ channels, Ca^{2+} influx and release, c-Jun NH2-terminal kinase, extracellular signal-regulated kinases, nuclear factor- κB and SIRT1 deacetylase (17). Therefore, although short-term exposure of β -cells to ROS might be beneficial in terms of promoting insulin secretion induced by glucose, chronic production of ROS and levels of ROS above a critical threshold might lead to β cell dysfunction and/or apoptosis and reduction of insulin secretion (18). Finally, oxidative stress has also been implicated in ovarian and testicular aging, perturbing cellular niche and down-regulating steroidogenesis (19).

On the other hand, during the last 5 years, multiple scientific data have forced an intense re-evaluation of the mitochondrial free radical theory of aging. Specifically, in a study by Van Remmen *et al.* genetic manipulations in mice that increased mitochondrial ROS and oxidative damage do not accelerate aging (20), while mice with increased anti-oxidant defenses do not present an extended lifespan (21). Additionally, ROS are necessary in various physiological body functions, including cell proliferation and metabolism, gene expression, immune function and reproduction (22). A protective action of ROS is seen in skeletal muscle, in case of increased energy production, typically happening after a high fat, high glucose meal, where functional insulin resistance is imposed to cells, through dismutated hydroxyl radicals that diffuse to the cell membrane to oxidize and block the insulin receptor, in order to avoid irreversible damages to the mitochondria, until the system recovers the ability to balance (23).

Taken all the above into account, ROS and oxidative stress are actively implicated in both physiological and pathological phenomena that take place in cells. Thus, it can be postulated that the primary effect of ROS is to enhance cell function and stimulate homeostatic mechanism. Across lifespan, mitochondrial damage and cellular stress occur, increasing in parallel levels

of ROS. Beyond a certain threshold, ROS lose their physiological and favorable functional role, promoting eventually senescence, apoptosis and aging. More studies are necessary to further investigate this dual role of ROS and establish definitive conclusion regarding their contribution to aging.

Future personalized anti-aging diagnosis and treatment: role of stem cells

The development of stem cell based therapies to combat the age-associated functional decline has recently attracted intense attention as their potency to differentiate and give rise to all cellular types provides novel opportunities for regenerative medicine. Stem cells are defined as pluripotent cells that possess both the abilities of self-renewal and differentiation toward numerous cell types, divided in to two main categories: embryonic stem cells (ESCs) that are derived from the inner cell mass of the blastocyst and have unlimited proliferative capacity (24) and adult stem cells that reside in tissues throughout the body such as the brain, bone marrow, pancreas, liver, skin and skeletal muscle (25) and are critical for tissue maintenance and regeneration.

It has been suggested that several aspects of mammalian aging correlate with an age-related decline of adult somatic stem cell function. Specifically, the decline of homeostatic and regenerative capacities of aging tissues has been attributed to degenerative alterations in tissue-specific stem cells, stem cell niches and systemic signals regulating stem cell functions. The fact that adult stem cells remain in tissues throughout life, renders them particularly sensitive to the accumulation of cellular damage, which can ultimately lead to cell death, senescence and loss of regenerative potential. In fact, it has been observed that stem cells in various aged tissues exhibit attenuated response to tissue damage, deregulated proliferation and reduced regenerative potential (26). This exhaustion of stem cells contributes to tissue dysfunction and to age-related diseases such as frailty, atherosclerosis, progressive Parkinson's disease, type 2 diabetes, anemia and malignancies (27). Aging of stem cells is affected by the dynamic interplay of diverse cell-intrinsic, environmental and systemic signals. The most prominent conserved cellular processes that drive stem cell aging and affect organismal lifespan include the accumulation of DNA damage, epigenetic alterations, loss of proteostasis, increased oxidative stress, mitochondrial dysfunction and deregulated extracellular signaling (26) (Fig. 1).

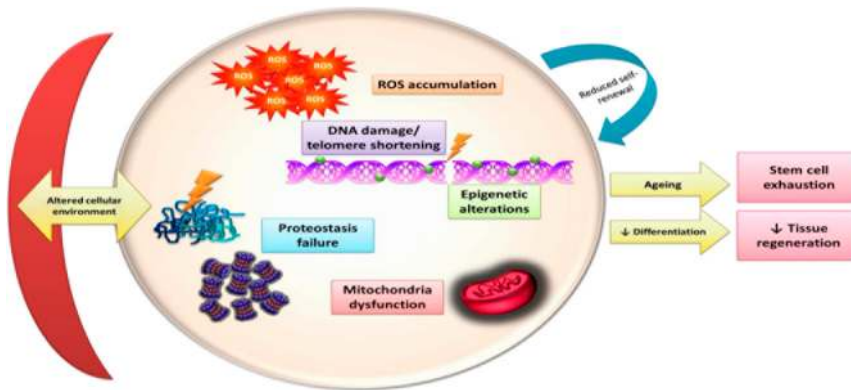


Figure 1

Schematic representation of the pathways that are involved in stem cell aging.

As stem cells persist throughout life in a quiescent state, they experience long-term exposure to genotoxic assaults during aging, leading to the accumulation of DNA damage. For instance, aged HSCs (hematopoietic stem cells) display elevated levels of DNA double strand breaks (28, 29, 30), while telomeres are shorter in aged hair follicle stem cells (31). The compromised capacity of aged stem cells to respond to and repair DNA damage also contributes to the accumulation of genotoxic lesions, leading to senescence or apoptosis of stem cells and to the subsequent dysfunction of aged tissues.

While genome integrity is vital for the survival of stem cells, proteome stability is equally critical for stem cell identity. The proteasome, which plays a pivotal role for the maintenance of proteostasis, is involved in the regulated degradation of normal as well as abnormal, oxidized, denatured or otherwise damaged proteins (32). Studies employing biobanks from donors of different ages, including centenarians and long-lived siblings, have demonstrated that healthy centenarians have an active proteasome (33), while treatments with various oxidants resulted in enhanced proteasome assembly and activities and increased cell survival (34, 35). Age-related dysfunction of proteasome is also heavily implicated in stem cell senescence, while proteasome activation has been shown to enhance stemness and lifespan of human mesenchymal stem cells (36).

Furthermore, ROS-mediated oxidative damage is thought to contribute to perturbed function and accelerated aging of stem cells. ROS levels are elevated in senescent mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs) and neural stem cells (NSCs), while ectopic induction of SIRT3 expression enhances the anti-oxidant activity of SOD2, subsequently improving the function of aged HSCs. Additionally, several studies have linked mtDNA damage, nutrient sensing and energy homeostasis to human stem cell aging. Conversely,

improved mitochondrial function has been associated with enhanced stem cell function and tissue regeneration in mammals. For instance, short-term (1 week at 20% restriction and 11 weeks at 40%) calorie restriction (CR) increases the numbers and function of skeletal muscle and intestinal stem cells potentially by elevating mitochondrial content and facilitating oxidative metabolism (37). Besides modulating mitochondrial function, CR also promotes proteostasis and scavenging of ROS and protects from DNA damage. In support, the experimental administration of rapamycin, a CR mimetic compound, inhibits mTORC1, promotes energy and protein homeostasis and decelerates stem cell aging (38, 39, 40).

Given the emerging evidence that aging may reflect an exhaustion of adult stem cells, the great interest in restoring adult stem cell function, to ameliorate age-related pathologies and rejuvenate aged tissues, is not surprising. Stem cells are considered to be very promising tools for several regenerative cell based therapies, not only because of their high proliferative and multi-lineage differentiation capabilities, but also because they can be obtained autologously, thus eliminating the need for immunosuppressive therapy to avoid the risks of rejection (41). It should be noted that MSCs are among the most promising candidates for personalized interventions to combat aging and the associated disease states. Studies suggest that MSCs after systemic transplantation, migrate to sites of injury and exert immunomodulatory effects, facilitating tissue repair via the production of growth factors and signaling molecules that guide resident tissue cell regeneration (41, 42). The clinical safety of MSCs is well accepted and is currently used to enhance tissue regeneration, to extinguish cancer cells, to treat vascular disorders and to regenerate cartilage and bone (41). Adipose-derived stem cells and their secretory

factors have also proven useful for the treatment of skin aging (43).

Stem cell based therapies also appear to have enormous clinical potential for the autologous treatment of age-related diseases. The exposure of aged human MSCs to proangiogenic growth factors *ex vivo*, increases their regenerative potential when transplanted into an infarcted rat heart (41). In addition, vascular endothelial cell precursors can be induced to proliferate *in vivo* and form new blood vessels in response to VEGF, alleviating atherosclerosis and improving recovery after myocardial infarction and stroke (44). Likewise, the systemic administration of growth factors restores the proliferative capacity of aged MSCs *in vivo*, sufficiently reversing the osteoporotic phenotype (41).

Furthermore, the transplantation of MSCs and NPCs engineered to produce growth factors such as BDNF (brain-derived neurotrophic factor), VEGF, GDNF (glial cell line-derived neurotrophic factor) and IGF-I (insulin-like growth factor 1) safeguards dopaminergic neurons and improves the functional deficits in rodent models of Parkinson's disease (45). Additionally, rodent Alzheimer's disease (AD) models receiving NSCs transplants in the hippocampus demonstrate increased neurogenesis and ameliorated cognitive function via a BDNF mediated response. Striatal injections of NPCs into HD rodents have pointed out the potential of stem cells to restore the functionality of medium spiny neurons in Huntington's disease by migrating to affected sites. Furthermore, the transplantation of GDNF-overexpressing NPCs has neuroprotective effects and promotes functional recovery in HD rodents. The systemic intraspinal injection of NPCs and MSCs ameliorates Amyotrophic Lateral Sclerosis (ALS) progression in rodents, while overexpression of GDNF, VEGF and IGF-I further enhances neuroprotection (45). Several studies have described some preliminary data reporting clinical benefit from autologous stem cell therapy in ALS patients (45). In these patients, stem cell delivery took place with different routes, including intraspinal and lumbar (45) or into the frontal motor cortex (46). The clinical safety of autologous stem cell interventions to treat ALS has been recently supported by studies describing the implantation of MSCs into the frontal motor cortex of 67 ALS patients (46) or into the dorsal spinal cord of 19 patients (47). Hence, even if stem cell therapy is still in a preliminary phase for clinical applications, it has demonstrated some very promising results in several age-related pathological processes.

Exploiting reprogramming techniques to derive iPSC cells from somatic patients' cells is a highly attractive

alternative method circumventing the limitations of aging adult stem cells for anti-aging approaches (48). For instance, the transplantation of patient-specific iPSCs into a rodent PD model results in their integration in the host tissue and a significant improvement of the disease phenotype (45). However, despite the promising results of iPSC therapies in animal models, there are substantial barriers that must be overcome before they become feasible in humans. The most principal obstacles involve the forced expression of transcription factors that are highly linked to oncogenicity, such as c-Myc and Klf4, and the use of viral vectors for gene delivery (48). The generation of senescence-resistant stem cells would be a more ideal approach for stem cell based therapies. Interestingly, the *ex vivo* rejuvenation of aged fibroblasts using an excisable vector for reprogramming has proven to be feasible (48). Further studies addressing the safety of iPSC-based therapies are needed before they are transitioned in clinical settings.

Understanding the molecular processes that govern stem cell self-renewal, proliferation and commitment to specific differentiated cell lineages is essential for the development of therapeutic interventions that can slow, or even reverse the functional decline in aging tissues by targeting the specific causes or enhancing repair processes to maintain healthy function. Additionally, developing methods to modulate them will be critical for the development of regenerative interventions to promote healthy longevity in a patient-specific basis.

Aging endocrine glands and anti-aging hormonal replacement

Aging pituitary

The hypothalamic–pituitary unit is a central regulator not only of all endocrine axes, but also of many systems and as such, it controls the body temperature, nutrient intake and energy balance, sleep and wake cycle, sexual behavior, reproductive cyclicity, water and electrolyte balance, stress adaptation, and circadian or ultradian cycles. As a result, the hypothalamus is the master regulator of homeostasis, as well as the source and the target of continual regulatory adjustments throughout aging.

Aging and negative feedback

With advanced age, the sensitivity of the hypothalamus to various feedback signals begins to decline. Thus, in the reproductive axis, responsiveness of the hypothalamus

to estrogen feedback gradually decreases with age, a decline restored with L-DOPA pretreatment, a D1receptor agonist and secretagogue of GnRH. In the major stress adaptive (adrenal) axis, decreased responsiveness (cortisol suppression) to dexamethasone has been noted. Furthermore, a reduced decline of GH to glucose load, due to gradual decline in growth hormone production by the pituitary gland, is a landmark of the aging somatotroph. These alterations led Dilman to propose over 40 years ago the neuroendocrine theory of aging postulating that the functional decline of the hypothalamus is due to a decrease in its sensitivity toward feedback control (49).

Many factors have been identified as either the cause or the consequence of age-related decline in functions and repair mechanisms. Activators/modulators of the aging process have been identified in the hypothalamus, such as mTOR, IKK-b/NF-kB complex and HIF-1a. These molecules play crucial roles in nutrient sensing, metabolic regulation, energy balance, reproductive function and stress adaptation.

In a recent study by Chen *et al.*, the role of succinate, one of the most prominent intermediates of the Krebs cycle in the aging process has been evaluated. Succinate oxidation in mitochondria provides the most powerful energy output per unit time. Extra-mitochondrial succinate triggers a host of succinate receptor (SUCN1 or GPR91)-mediated signaling pathways in many peripheral tissues including the hypothalamus. One of the actions of succinate is to stabilize the hypoxia and cellular stress conditions by inducing the transcriptional regulator HIF-1a. Through these actions, it is hypothesized that succinate has the potential to restore the gradual but significant loss in functions associated with cellular senescence and systemic aging (50).

Aging and circadian rhythms

As we mentioned above, in aging, a decrease melatonin secretion has been found, with reduced expression of specific genes in peripheral clocks and disruption of neural circuits in the hypothalamus, resulting to less total sleep time, poorer sleep efficiency, increased sleep latency, increased night-time awaking, excessive daytime sleepiness with increased daytime naps. This disruption of the fine tuning between darkness/light, central and peripheral 'clock system' leads to decay of the circadian system, alteration of hormones and metabolites levels and abnormal nutrient intake with detrimental effects on health. A plethora of the most common health disorders in the elderly such as neurodegeneration, insulin resistance,

diabetes, obesity and cancer may be attributed to the above-mentioned disruption of the circadian rhythms (51).

Aging and the hypothalamic–pituitary–peripheral axes

In the aging subjects cortisol secretion is characterized by increased late night levels and restricted period of low cortisol secretion. Data indicate increased pituitary responsiveness to CRH, while adrenal responsiveness to ACTH seems to be lower in aged males than that in aged females (52). Disruption of diurnal cortisol rhythm is more pronounced in patients with dementia together with decreased DHEAs levels. It has been demonstrated that cortisol induces apoptosis and death of hippocampal neurons, while DHEAs has a protective effect. Considering that dementia is characterized by a decreased number of hippocampal neurons, there is a possibility that adrenal axis alteration in the elderly has a role in the pathophysiology of dementia in this cohort (53).

The hypothalamic secretion of GHRH from the arcuate nucleus is lower in elderly, while somatostatin secretion from the paraventricular nucleus is increased with net result alteration on growth hormone. Growth hormone secretory profile from the somatotroph exhibit reduced frequency and lower amplitude of secretory pulses. Moreover, metabolic factors have a key role in GH regulation and thus, increased nutrient intake leads to increased levels of free fatty acids in the circulation which stimulate insulin secretion from the pancreas. Hyperinsulinemia and increased free fatty acids inhibit GH secretion. In addition, they reduce the production of IGFBP-1 from the liver, exacerbating the negative effect on GH secretion. As a result of the above-mentioned changes IGF-1 levels decline with age (54).

Thyroid axis in the elderly is characterized by lower sensitivity of thyrotrophs to TRH in men, possibly resulting by lower 24-h rhythmicity of TRH or increased somatostatin secretion. Aged women have higher TSH levels and both sexes demonstrate lower T3 and increased anti-thyroid antibodies concentration (49).

Aging and water balance

Aged individuals exhibit 20% reduction in maximum urine osmolality, 50% decrease in the ability to conserve solute, 100% increase in minimal urine flow rate, nocturnal polyuria and frequent episodes of hypo-hyponatremia.

Vasopressin, acting via different receptor subtypes, is the key regulator of plasma and urine osmolality. Binding

to its specific V2 receptor stimulates cAMP/protein kinase A (PKA) axis resulting in a significant increase of the apical expression of the vasopressin regulated water channel aquaporin (AQP)-2 thus regulating water permeability in the collecting duct. Interestingly there is a distinct circadian rhythm in vasopressin secretion, with peak concentrations about midnight, a decrease during daytime and minimum levels in the afternoon. An attenuated vasopressin response is also noted upon water ingestion.

Vasopressin secretion is increased in the elderly and is not normally suppressed by water ingestion. Simultaneously, vasopressin action in the collecting duct is subnormal (indicating receptor defect leading to vasopressin resistance) leading to increased urine flow rate, which together with the decreased thirst exhibited in this age group results in impaired ability to conserve water and reduction of plasma volume (55). Furthermore, decreased renin-aldosterone activity exists in aged individuals leading to abnormal movement of solutes (56). The final result of these abnormalities is higher sensitivity to water overload or dehydration, and increased risk for developing hypo- or hypernatremia.

Endocrine changes to the elderly: to treat or not to treat?

To consider treating endocrine alterations in the elderly we have to answer the question if and to what extent these changes are adaptive or maladaptive. Also we have to note that many comorbidities can affect the pituitary function during aging such as obesity, diabetes mellitus, reduced nutrition, systemic illness and medication. On clinical grounds the only relevant therapeutic intervention is the prevention and the treatment of hyper-, hyponatremia and dehydration. We must also have in mind when dealing with an endocrine abnormality in an aged individual that previously undiagnosed true hypopituitarism can occur in this population and of course appropriate hormone replacement is the treatment of choice.

Aging thyroid gland

The process of aging brings about changes in the thyroid gland that are part of an adaptive mechanism maintaining homeostasis in this age group. In areas with adequate or high iodine intake, TSH levels are generally found to be high and positively associated with age, in contrast to

mildly or moderately iodine-deficient areas where TSH levels have been reported lower and negatively related to age (57, 58). The latter likely indicates an autonomous function, corroborated by the finding of a regionally positive association between free thyroxine and age. Thus, differences between age and thyroid function, in relation to iodine intake in the past, iodine status at present and the historical iodine status of a population, must be taken into account, in order to ensure the proper establishment and evaluation of TSH reference values (59).

When the thyroid gland is underactive, fewer hormones are produced which may not be sufficient to maintain an active metabolism, hence symptoms such as weakness, fatigue and weight gain are frequently manifested. In a computer based analysis of untreated spontaneous autoimmune hypothyroidism, a four-fold difference in average serum TSH levels between the young and old was observed. For the same degree of thyroid failure, the serum TSH is lower among the elderly, due to a decrease in the hypothalamic-pituitary response to low serum T4, with instances of strongly increased serum TSH being due to severe hypothyroidism (60). Furthermore, log TSH exhibits an inverse correlation with age, while serum T4 does not show any correlation. It is crucial to note that, as it presently stands, the TSH upper reference limit tends to be too low in the elderly (and notably in women and white individuals), possibly leading to unnecessary or even harmful therapy. A decline in thyroid activity in aging may cause a compensatory increase in cellular deiodinase (DIO) I activity and thyroid hormone metabolites, which probably, through non-genomic pathways, counteracts the aging-related metabolic disturbances (61). Therefore, physicians should scrupulously weigh up age-related hormonal changes in their patients, bearing in mind that if thyroid values appear low, this does not necessarily mean that the patient should be immediately medicated.

Hypothyroidism treatment in the elderly

Over the last few decades, a constant increase of thyroid autoimmunity and subclinical hypothyroidism (SCH) of up to more than 20% in women older than 65 years old has been reported (62). In this context, SCH commonly manifests, when peripheral thyroid hormone levels are within the normal reference laboratory range but serum TSH levels are high, a condition which may progress to overt hypothyroidism and lead to impaired cardiac function (63). According to the recent Guidelines, patients with persistent SCH, whose TSH levels are

greater than 10IU/L and who test positive for anti-thyroid antibodies and/or are symptomatic, should be treated with l-thyroxine (LT4) to reduce the risk of progression to overt hypothyroidism, decrease the risk of adverse cardiovascular events and improve their quality of life (64, 65). This is also substantiated by evidence showing that SCH is associated with an increased risk of cardiovascular mortality particularly in patients with a TSH concentration of 10IU/L or above (66). Hence, among elderly subjects with SCH, approximately 80% have a serum TSH of less than 10IU/L: in these patients, the decision to initiate treatment should be tailored specifically to each individual.

Additionally, in a recent meta-analysis including 61 studies type 2 diabetes mellitus (T2DM) was clearly associated with SCH when T2DM patients were compared with the healthy population. Consequently, appropriate individualized treatment should be provided to T2DM patients who concurrently have SCH as they may be at increased risk for diabetic complications (67, 68). In an analysis of the United Kingdom General Practitioner Research Database to identify individuals with new SCH (serum TSH levels of 5.01–10.0IU/L and normal free thyroxine levels, FT4) performed separately for younger (40–70 years) and older (above 70 years) persons, it was demonstrated that LT4 treatment was associated with fewer ischemic heart disease events in only younger but not in older individuals (69). These results are corroborated by another recent study in which treatment of 136 patients (median age 73.6 years) with LT4 during a median follow-up time of 5.6 years did not exhibit any increased risk for all-cause mortality or major adverse cardiac events, defined as cardiovascular death, fatal or nonfatal myocardial infarction and stroke (70).

In a cohort analysis of 643 individuals aged 85 years who were assessed in their own homes for health status and thyroid function and followed for mortality and disability for up to 9 years, all-cause mortality was associated with baseline serum rT3 but not FT4 or TSH. The study did not show worse survival for patients with both subclinical hypothyroidism and subclinical hyperthyroidism over the 9 years in comparison with their euthyroid peers, with higher serum TSH levels prognosticating better outcomes (71). Simultaneously, in euthyroid old men (aged 70–89 years), higher FT4 levels have been associated with all-cause mortality independently of cardiovascular risk factors and comorbidities (72).

It has been reported that extreme longevity is associated with high TSH levels, indicating that shifts to higher concentrations with age may produce a continuum

resulting in populations of centenarians; with a persistent inverse correlation between TSH and FT4 pointing to the possibility that changes in negative feedback might be involved (73). Interestingly, the offspring of centenarians also have elevated serum TSH as compared to controls; moreover, they also show specific single nucleotide polymorphisms (SNPs) in the TSH receptor (TSHR) gene that are associated with this phenotype (73). It is therefore evident that a heritable phenotype characterized by raised serum TSH marks human longevity. Moreover, carriers of SNPs in the TSHR gene likely have higher serum TSH, this possibly contributing to decreased thyroid function and longevity (73). Thus, aging is associated with increased serum TSH with no change in FT4 concentrations, the largest TSH increase being seen in persons with the lowest TSH at baseline (74). The latter implies that the TSH increase arises from age-related alteration in the TSH set-point and is not due to minimal thyroid disease.

Given that there is a lack of large studies providing evidence of any benefit of LT4 treatment in the elderly, as stratified according to age and TSH, treatment recommendations should be tailored on an individual basis, taking into account not only the degree of TSH deviation but also age and comorbidities (75). In this regard, patients aged older than 65 years who have a sustained TSH >5 U/L should be carefully assessed and treatment should not be initiated unless the patient displays an array of clinical symptoms, increased TPOAB, and comorbidities such as T2DM and secondary hypercholesterolemia. In the older population, above 85 years old, or in the centenarians a 'wait-and-see' strategy is advisable, taking into account the increased upper limit of the TSH reference interval from 6.45 to 7.55 U/L, the general condition of the patient and the potential coexistence of heart disease (76).

Hyperthyroidism in the elderly

The thyroid morphologically and anatomically changes during the process of aging, with increased nodularity being observed. Moreover, the gland is likely to reside at a more caudal position in the neck in the elderly as compared to younger individuals, which means that clinicians need to take special account of this fact when surgery is planned (77, 78).

In the elderly, hyperthyroidism is mainly due to autonomous thyroid nodules while in about 20% of the hospitalized patients hyperthyroidism may occur following a contrast radiography procedure. Among older aged patients who develop hyperthyroidism fewer hyperadrenergic signs are noted; on the other hand,

elderly hyperthyroid patients tend to have an elevated incidence of cardiac arrhythmias and weight loss as well as, not infrequently, depressed mood, with the commonest clinical features being weight loss (83%) and atrial fibrillation (60%). In general, clinical signs in older people are mild, and even often absent, and are caused by age-related changes: namely, decreased cellular uptake of thyroid hormone along with changes in thyroid hormone regulation of gene expression, all of which possibly explain the fewer manifestations of hyperthyroidism in this age group (79).

In elderly patients with endogenous subclinical hyperthyroidism and TSH between 0.1 and 0.4 IU/L, persistence of SCH for many years is usual, though progression to manifested hyperthyroidism is rare (approximately 1% per year) and spontaneous TSH normalization is fairly common (80).

Treatment options for hyperthyroidism in older patients include anti-thyroid drugs, radioiodine therapy and surgery (81). Interestingly, as observed in a prospective observational population-based study of 1036 subjects treated with anti-thyroid drugs or radioiodine, mortality was only increased during periods of thionamide treatment and after radioiodine not resulting in hypothyroidism (82).

Aging female

Ovarian aging

Physiologic systems, including endocrine, have substantial reserves in younger individuals. Aging and intercurrent pathologies gradually eliminate these reserves (83). A perfect example of this is menopause. Menopause is a hallmark of a woman's life, taking place approximately during the sixth decade of her life. It is characterized by depletion of ovarian follicles and huge hormonal alterations that are accompanied by significant clinical consequences (84).

As women age, ovarian follicles function less well, frequency of ovulation decreases and serum estradiol levels are lower than younger individuals. Simultaneously, while LH seems to remain unchanged, FSH levels increase premenopausally, a sign predicting reduced ovarian reserve. Eventually, follicular activity ceases, estrogen levels fall to postmenopausal values and gonadotropins rise above premenopausal concentrations (85).

For many years, the prevailing view was that menopause resulted from exhaustion of ovarian follicles. However, it is now widely accepted that derangements in

central nervous system and in the hypothalamic–pituitary axis contribute to menopause equally. Specifically, hypothalamic adaptations during aging favor augmented GnRH release (49). This enhanced GnRH release was also found in gonadectomized animals, creating gonadotrope ‘castration cells’ and pituitary pericyte hyperplasia (86).

Additionally, the ‘free radical theory of aging’ was also implicated in the pathogenesis of ovarian aging. Analytically, serum concentrations of inflammatory cytokines and pro-oxidant biomarkers such as oxidized lipoproteins (ox LDL), 4-hydroxynenal and MDA were found to be higher in postmenopausal women than in premenopausal women (87). In contrast, GSH and glutathione transferase (GST), which are effective in the removal of free radicals, are reduced in oocytes with age (88). Similarly, Carbone *et al.* demonstrated that follicular fluid from older women exhibited a reduced level of glutathione transferase and catalase activities and a higher level of superoxide dismutase (SOD) activity (89). Taken together, the results indicated that reproductive aging is accompanied by a change in the anti-oxidant enzymatic pattern that could impair ROS scavenging efficiency in the follicular environment.

Finally, environmental stimuli play a decisive role in the evolutionary process of ovarian aging. A vivid example of environmental factors affecting female reproductive system is advanced glycation end products (AGEs), commonly found in tobacco and foods, high in protein and fat, cooked in high temperatures (90). Physiologically, AGEs are present in normal ovarian tissue, while RAGE was highly expressed in the ovary being present in granulosa cells, theca interna, endothelial and stromal cells (91). Potential accumulation of these compounds in the ovary across lifespan may initiate adverse molecular intraovarian events, such as compromised efficiency of vascularization and activation of oxidative stress response through RAGE interaction, and ultimately trigger ovarian dysfunction (92).

Aging of the gonadotropic axis is important clinically because sex-steroid privation adversely affects muscle and bone mass, visceral adipose tissue accumulation, insulin sensitivity, LDL metabolism, and possibly mood, libido, cognition, memory and quality of life. Postmenopausal women experience vasomotor symptoms, psychological instability, sleep disorders and have a higher risk of cardiovascular disease, osteoporosis, mood disorders, cognitive decline as well as premature death (93, 94). Indeed, as reported in a recent meta-analysis (95), younger age at menopause is associated with a higher risk of coronary heart disease (CHD: RR=1.50; 95%

CI: 1.28–1.76), of stroke (RR=1.23; 95% CI: 0.98–1.53), of cardiovascular mortality (RR=1.19; 95% CI: 1.08–1.31) and of all-cause mortality (RR=1.12; 95% CI: 1.03–1.21). All the above indicate that menopause, a natural consequence of aging of the reproductive axis, constitutes a standpoint in a woman's life that requires intense medical attention, in order to alleviate clinical sequelae and ensure a good quality of life.

Anti-aging measures: fertility preservation

During the reproductive years, folliculogenesis, steroidogenesis, and the production of proteins such as inhibin occur in response to the gonadotropins LH and FSH. As women age, there is a reduced pool of follicles, reduced inhibin concentrations and, through feedback, FSH concentrations rise. The increased FSH is most evident in early cycle, reflecting the diminished reserve of follicles. All the tests of ovarian reserve, as outlined below, attempt to evaluate this decreased reserve.

Tests of ovarian reserve

Day 3 FSH: In young women it is less than 10 IU/L. FSH concentration >10 has a high specificity (80–100%) but poor sensitivity (10–30%) for predicting poor response to ovarian stimulation. FSH between 10 and 15 is suggestive of low reserve. Pregnancy, using the woman's own oocytes, is unlikely when FSH is >20. As folliculogenesis is accelerated with aging, FSH needs to be interpreted with caution if the day 3 estradiol exceeds 60 pg/mL (96).

Clomiphene challenge tests: Clomiphene has both estrogenic and anti-estrogenic properties. The latter stimulates hypothalamic GnRH release, translating into higher FSH and LH concentrations that further induce follicular development. Hence, it has been used as a test of ovarian reserve, involving oral administration of 100 mg clomiphene citrate during days 5–9 of menstrual cycle and with measurement of gonadotropins and estradiol on day 10. An exaggerated FSH response (usually with a lower estradiol response) has been interpreted as indicative of poor ovarian reserve. Meta-analyses of non-randomized studies have concluded that it performs similarly to day 3 FSH for predicting the ability to achieve pregnancy in women undergoing treatment for infertility (97).

Anti-Mullerian hormone (AMH): AMH is a member of the TGF- β family, secreted by granulosa cells of preantral and early antral follicles, declining with age. Values below 0.7 ng/mL have been associated with decreased fecundability in natural cycles and poor response to

stimulation in assisted reproduction (98). It is unaffected by cycle day. Values 0.2–0.7 have a sensitivity of 40–97% and specificity of 78–92% in predicting poor ovarian response to ovarian stimulation (98).

However, low AMH concentrations do not translate into lower pregnancy and live birth rates in women <36. While lower values represent a diminished pool of follicles, the quality of the remaining oocytes is unaffected and hence the favorable outcomes.

Antral follicle count (AFC): Follicles of 2–10 mm are counted in early follicular phase; this correlates with remaining primordial follicles histologically and if low is predictive of poor ovarian response to stimulation.

Ovarian reserve tests, which are all measures of oocyte quantity, correlate well with each other (99). Maternal age is the best predictor of oocyte quality and determines the rate of aneuploidy. In a commentary on oocyte competency, Keefe *et al.* implicated the following in age-related oocyte dysfunction: mitochondrial and meiotic spindle dysfunction, free radical production and telomere attrition. They proposed a telomere theory of reproductive aging and suggested two hits to telomeres. The first may occur in early development with attrition during the mitotic division of oogonia. The second may result from the prolonged interval between germ cell formation and ovulation, when reactive-oxygen species, inevitable by-products of metabolism, further shorten telomeres. They further suggested that polar body telomere content may be a promising biomarker (100).

Methods of fertility preservation: reproduction in patients with forced ovarian aging

In women, since there are only a finite number of germ cells, any insult may result in complete loss of ovarian germ cells or may decrease the available number, with early menopause ensuing. The damage inflicted is dependent on the age of the subject (those that are younger have a greater reserve), and the treatment protocol (regimen containing alkylating agents lead to depletion in a dose-dependent way). Thus, a recent meeting of international experts, convened to discuss cancer and fertility preservation, recommended the following: 'International guidelines recommend that physicians discuss, as early as possible, with all patients of reproductive age their risk of infertility from the disease and/or treatment and their interest in having children after cancer, and help with informed fertility preservation decisions' (101).

Among the existing technologies of fertility preservation, embryo cryopreservation after controlled

ovarian stimulation is best for women with available partners. Random start protocols are acceptable, as uterine receptivity is not an issue. Success rates are high with survival and implantation rates of 90% and 30% respectively. Aromatase inhibitors may be used for stimulation in breast cancer patients (102). Additionally, cryopreservation of mature oocytes after ovarian stimulation is another fertility method, which is no longer considered experimental; high survival and pregnancy rates of 90% and 40–55% have been reported in egg donation programs. It is the appropriate method for post-pubertal subjects. While only a few live births have been reported, *in vitro* maturation of immature oocytes, which are then cryopreserved when mature, may also be considered, especially if delay in cancer treatment is not possible (103). Finally, ovarian tissue cryopreservation is another option for fertility preservation. While still considered experimental, removal and cryopreservation of cortical ovarian tissue with subsequent transplantation, has been performed in many centers. It is the best option for pre-pubertal girls, and multiple pregnancies have resulted from this treatment from several centers, some spontaneous and some after *in vitro* fertilization. This method is likely to be adopted by many other countries and centers (104).

Apart from all the above, more and more fertility methods are emerging. These include ovarian follicle culture *in vitro*, engineering of an artificial ovary comprising ovarian follicles embedded in a viable matrix that can be transplanted back into the recipient, search for the possible existence of oogonial stem cells and use of induced pluripotent stem cells. Furthermore, fetoprotective adjuvant therapy with GnRH agonists has been used for many years to down regulate the hypothalamic–pituitary axis and shut off gonadal function, in patients undergoing gonadotoxic therapeutic regimens. Meta-analysis showed some protective effects in breast cancer patients but a recent study in lymphoma patients did not. Other agents, inhibiting apoptosis are under investigation (103).

Anti-aging measures: menopausal hormone therapy (MHT)

Permanent cessation of menses and concomitant hormonal alterations are accompanied by a plethora of clinical symptoms and comorbidities that jeopardize women's quality of life. Attempts to ameliorate estrogen deficiency and its consequences, via menopausal hormone therapy

(MHT), have however courted significant controversy due to its increased risk for malignancy and vascular events.

The indications of MHT are the management of menopausal symptoms, urogenital atrophy and the prevention of osteoporosis in symptomatic women. Menopausal symptoms include hot flushes and night sweats, sleep disturbances, mood swings, anxiety, fatigue, muscle and joint pain and headaches. MHT regimens should not be prescribed to all postmenopausal women; the decision to treat, the type, the dose, the mode and the route of administration of MHT need to be individualized (105). The type of menopause, the menopausal age as well as the presence of other comorbidities and risk factors will determine the most appropriate regimen (105). Available regimens differ with respect to the dose of estrogen, type of progestogen, mode of delivery (i.e. continuous combine vs cyclical administration) and route of delivery (i.e. transdermal vs oral) (105).

MHT and ischemic heart disease

Menopause is a natural hormonal transition that causes adverse effects on the cardiovascular system (106). Estrogen decline affects both directly and indirectly the vasculature, leading to the development of atherosclerosis (106). Beyond menopause, increasing age is associated with physical inactivity, mood instability and sarcopenia, resulting to weight accumulation and obesity, further exacerbating the atherosclerotic process (106). The formation of atherosclerotic plaques increases the risk of ischemic heart disease (IHD) and stroke (106). Recent research indicates that vasomotor symptoms may represent an independent risk factor of cardiovascular disease (107).

The effect of MHT differs according to the time of initiating treatment following the last menstrual period. Younger women, who are closer to their final menstrual period, are favorably affected by the intake of MHT, showing an improvement of blood lipids, insulin sensitivity, body composition, arterial stiffness and chronic inflammation (108). On the other hand, older postmenopausal women have already developed subclinical vascular disease with mature atherosclerotic plaques (108). These plaques may be destabilized by the intake of MHT, increasing thus the risk of developing an acute thrombotic event.

Appropriate administration of MHT has been linked to clear cardiovascular benefits. A significant reduction in rates of CHD by 18–54% as well as an increase in life expectancy by 12–38% has been reported by a nation-wide study in Finland (109). The same study (109) reported a

Table 1 Individualization of menopausal hormone therapy (MHT) according to cardiovascular risk.

High cardiovascular risk: NO MHT

- Age >60 years
- Menopausal age >10 years
- Long standing poorly controlled diabetes mellitus
- Clinically overt cardiovascular disease

Intermediate risk: transdermal MHT

- Menopausal age 5–10 years
- Dyslipidemia
- Obesity
- Smoking
- Hypertension
- Diabetes
- Low risk: any type of MHT

linear association of the cardiovascular benefits with the duration of MHT use, during a follow-up period of 15 years. A recent Cochrane review (110) showed that the effect of MHT depends on the timing of initiating treatment. Women starting MHT within the first decade following the menopausal transition had up to 48% decrease in the incidence of CHD and up to 30% reduction in mortality (110, 111). In support of these findings, the ELITE randomized-controlled trial (Early vs Late Intervention Trial with Estradiol) showed that estradiol therapy slowed the progression of subclinical atherosclerosis as compared to placebo, when administered in early but not in late postmenopausal women (menopausal age <6 years vs ≥10 years) (112). The individualization of MHT in the context of IHD is presented in Table 1.

Menopausal hormone therapy and breast cancer

Older epidemiological studies and randomized clinical trials linked MHT with a small but significant risk of breast cancer, with a relative risk between 1.25 and 1.35 (113, 114, 115, 116). Data from the Million Women Study reported a higher risk of breast cancer (RR=1.66), evaluating however an heterogeneous population of women with a non-randomized study design (117). Finally, the estrogen-only arm of the WHI study (Women's Health Initiative) reported that 7 years of unopposed estrogen therapy is associated with a lower breast cancer risk (RR=0.77) (118).

Customization is essential to minimize the MHT-associated risk of breast cancer in the clinical setting. In this aspect, evaluation of the patient, as well as of the MHT regimen is necessary (119, 120) (Table 2). Characteristics of the MHT regimen which may affect the risk of breast cancer, include: (1) estrogen monotherapy vs estrogen-progestogen use, (2) duration of use, (3) type of progestogen, (4) mode of hormone administration

(sequential vs continuous) and (5) possibly dose of MHT (105).

Menopausal hormone therapy and thrombosis

Oral MHT slightly increases the risk of stroke (RR ranges from 1.3 to 1.5) (121) and the risk of venous thromboembolic events (VTE) by 2–3 fold (122). Possible pathophysiological mechanisms include induction of clotting factors through the first pass effect in the liver, as well as plaque destabilization, in case of stroke (121). The absolute risk is negligible in young, recently postmenopausal women; however, the risk increases progressively with age and the presence of risk factors, like diabetes, obesity, hypertension, dyslipidemia, left ventricular hypertrophy, atrial fibrillation, immobilization or genetic thrombophilia (121). Large prospective epidemiological studies have shown that low-dose (E2 ≤50µg) transdermal MHT does not increase the thrombotic risk (122). Furthermore, the thrombotic risk may depend on the progestogen in the MHT regimen (122, 123, 124). Table 3 presents the basic principles that should be considered when prescribing MHT in the context of VTE.

In conclusion, MHT is an effective and safe treatment for the management of menopausal symptoms, urogenital atrophy and the reduction of the risk of osteoporotic fractures. When administered early after the menopause, within the so-called 'window of opportunity' (125), MHT can also be cardioprotective. The duration of MHT is defined by the needs and the risks of the woman on an individual basis.

Anti-aging measures: role of DHEA replacement

Synthesis and biological effects of DHEA and DHEAS

DHEA and DHEAS are primarily synthesized and secreted from the reticular zone of the adrenal cortex in response to stimulation by adrenocorticotrophic hormone (ACTH). They have a weak androgenic effect but they are considered as precursor hormones further metabolized into potent androgens and estrogens. DHEA and DHEAS are synthesized quantitatively, the most, from all adrenal steroids. DHEAS has a long half-life and thus ensures a stable concentration in the blood and without diurnal variations. DHEA is also synthesized in the ovaries. As ovaries do not possess enzyme DHE-sulfotransferase, DHEAS is most exclusively synthesized and secreted from the adrenal cortex. DHEA is further

Table 2 Customization of menopausal hormone therapy (MHT) in the context of breast cancer.

<p>1. Risk factors which interact with MTH</p> <ul style="list-style-type: none"> • Body weight • Alcohol consumption • Mammographic density <p>2. Risk factors which do NOT interact with MHT</p> <ul style="list-style-type: none"> • Family history of breast cancer • Low parity • Breast surgery for benign condition 	<p>➤ Customization of MHT regimen</p> <ul style="list-style-type: none"> • Lowest effective estrogen dose • Lowest breast exposure to progestogen (vaginal route/sequential regimens should be preferred) • Natural progesterone/dydrogesterone or SERMS for endometrial protection. Sequential mode of administration • Annual cost-benefit analysis and estrogen dose re-setting
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metabolized to androstenedione that could be aromatized to estrone. Although DHEAS and DHEAS are secreted in high concentrations, androstenedione is qualitatively important since it is most commonly converted into testosterone in peripheral tissues (126, 127).

Since adrenal androgens are precursor hormones, their biotransformation into active androgens and estrogen depends on the level of expression of the different enzymes in different cells. This ensures that androgen and estrogen sensitive tissues have some control over the local concentration of sex steroids, depending on the current needs. This intracrine mechanism provides reduction of the exposure of other tissues to androgens and estrogens thereby reducing the possibility of adverse effects (127). It is shown that an increased concentration of DHEAS in women is associated with a higher prevalence of cardiovascular disease. A possible explanation is that elevated levels of DHEA and DHEAS, as is the case in women with hyperandrogenism, can act as the activators of translocation of the glucocorticoid receptor (GR) in the nucleus, which lead to a change in the functional characteristics of GR (128).

DHEA and DHEAS have highest concentrations in the third decade of life, and that is followed by a gradual decline. By the time of menopause, DHEA concentration is reduced by 60%, and in the eighth and ninth decade of life is lower by 80–90% of the maximal concentrations (129). This period of declining of adrenal androgens is

called ‘adrenopause’ despite the fact that cortisol does not decrease but rather increase with aging (130). Adrenopause is independent of menopause and occurs in both sexes as a gradual process with the similar period of the occurrence and duration. It is assumed that the reduction of 17,20-lyase activity is responsible for the progressive reduction of DHEA and DHEAS with aging (131), although other factors like the decrease of the volume of the reticular zone or decrease of IGF-1 and IGF-2 concentrations could influence this process (132).

In recent years there has been a change in the concept that the synthesis of adrenal androgens gradually decreases with aging (133). It was observed that despite the overall reduction in the concentration of DHEAS, in 85% of the analyzed women adrenal androgen synthesis actually grow during the menopausal transition. The increase was attributed to the adrenal and not to the ovarian production of DHEAS confirmed in both women with ovaries and in those who had undergone ovariectomy (134).

DHEA exerts its effects by a metabolic transformation into androgens and estrogens, acting through their specific nuclear receptors. It could activate numerous signaling pathways as well through specific membrane, cytosolic and nuclear receptors in the endoplasmic reticulum. In addition to the effects mediated over the estrogenic and androgenic receptors, the direct effect of DHEA through G-protein-coupled membrane receptors on the activation of endothelial NO synthesis (eNOS)

Table 3 Individualization of menopausal hormone therapy (MHT) in the context of thrombotic risk.

<p>Screening for risk factors</p> <p>Personal or family history of VTE</p> <p>Increasing age</p> <ul style="list-style-type: none"> • Increasing menopausal age • Obesity • Immobilization • Diabetes mellitus • Smoking <p>Choice of MHT regimen</p> <ul style="list-style-type: none"> • In women with no risk factors, absolute risk is very small and any type of MHT can be chosen • In women with risk factors for VTE, low dose transdermal estradiol ($\leq 50 \mu\text{g}$) is recommended, in combination with micronized progesterone, preferably through the vaginal route
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(eNOS/cGMP signaling pathway) is increasing the synthesis of nitric oxide (NO) in endothelial cells (135). It indicated an inhibitory effect of DHEA on cell proliferation and apoptosis of endothelial and vascular smooth muscle cells which takes place independent of the estrogen and androgen receptors (136).

DHEA and fertility

There is an increase of infertility rates in aging women, and that is accompanied with the increased risk of miscarriage or birth of offspring with Down syndrome. In women with reduced ovarian reserve, different regimens of DHEA were applied with some of them showing an improvement in pregnancy rates (137). In the study on women labeled as 'poor responders', daily administration of 75 mg of DHEA during three months led to an increased number of mature oocytes and a significant reduction in the concentration of hypoxia inducible factor 1 (HIF-1) in the follicular fluid (138). This is leading to the conclusion that DHEA could influence on the follicular microenvironment. Moreover, when DHEA was administered in women with different causes of infertility including age, it led toward improvements in the number of oocytes retrieved and increased rate of successful fertilization (139).

Possible explanation of such an effect of DHEA can be found in the effect on the theca cells that have a significant role in the structural and hormonal support for the developing follicle. Namely, theca cells are the main cells in the ovaries involved in the production of DHEA and androgens. DHEA, synthesized in the theca cells, is converted into androstenedione, which is transported to the granulosa cells and further converted into estrone and estradiol 17 β (140). It is equally important that DHEA synthesized in the theca cells stimulates PPAR α production (141). Reducing the expression of PPAR α in aging cells is probably sufficient to initiate subsequent cytoplasmic dysfunction seen in aging oocytes. Delta-9 desaturase is one of the key enzymes in the regulation of PPAR α . Low concentration of this enzyme led toward an increased production of ceramides. In the murine model it was demonstrated that ceramides were present in all cumulus cells of aged mice and when they were transported from cumulus cells to oocytes, apoptosis was induced (142). Inhibin B secreted from developing preantral follicles could represent a significant factor in these processes. Inhibin B has a paracrine role and stimulates the production of androgens in ovarian theca cells (143), a necessary step to stimulate the production of DHEA in the theca cells.

Thus, therapeutic administration of DHEA could have a positive effect on the down-regulation of the process of atresia, and alleviation of oxidative stress in the ovaries potentially contributing to a better folliculogenesis (139).

The aging hyperandrogenic woman: does she need special care?

The most common hyperandrogenic states in aged or postmenopausal women are the polycystic ovary syndrome (PCOS) (still present in the fertile age) and the classic or non-classic forms of congenital adrenal hyperplasia (CAH/NCAH).

PCOS is intrinsically associated with several metabolic derangements, chiefly insulin resistance, excess weight or obesity and metabolic disorders. An unanswered question is whether or not this dysmetabolic pattern may confer some specificity to the postmenopausal woman with PCOS. An important issue is that, after menopause, PCOS women still have elevated androgen levels (144, 145), which in turn may have some negative impact in favoring metabolic alterations and, possibly, an increased cardiovascular (CV) risk. Preliminary data from a new rat model of postmenopausal PCOS have in fact shown that they are characterized by higher insulin and non-fasted glucose levels than aged controls (146).

In women with PCOS a significant association between the metabolic syndrome and hyperandrogenemia (defined by increased testosterone and/or androstenedione and/or free testosterone) may significantly predict the dysmetabolic phenotype, as well as insulin resistance, in women with PCOS (147, 148, 149). Other studies have also shown that the prevalence and incidence of impaired glucose tolerance states are significantly higher in women with PCOS than in non-affected control women (150). In addition, it has been calculated that the relative risk of conversion from normoglycemia to IGT or T2D in PCOS tends to be significantly accelerated with increasing age in these women (151). We can therefore conclude that PCOS women are characterized by an intrinsic higher susceptibility to develop altered glucose intolerance states and T2D with increasing age. Notably, there is some indirect evidence that this may be favored by the combination of increased androgen blood levels and excess body weight.

Whether CV diseases are more common in these women is still under debate, in spite of the fact that PCOS by itself is strongly associated with multiple risk factors, partly independent of obesity, but strictly interconnected with the presence of insulin resistance. One major

scientific limitation in this area is the fact that women are often under-represented in CV clinical trials as well as in epidemiological studies (152). Nevertheless, there are studies showing that, in addition to CV risk factors, evolution indexes of atherosclerotic disease such as coronary artery calcification as well as increased carotid-intima-media thickness were significantly more frequent in women with PCOS than in control women without it (153). Intriguingly, PCOS women do not seem to have markedly higher than average mortality from CV diseases, even though the PCOS by itself is strongly associated with T2D, lipid abnormalities, low-grade inflammation and other CV risk factors (154). On the other hand, it has been also shown that postmenopausal women with a history of irregular menses plus elevated androgens (both cardinal features of PCOS) were associated with more angiographic coronary artery disease and worsening CV event-free survival (155). At variance, a large retrospective study from the United Kingdom did not find any association between all-cause mortality (including CV disease mortality) in women with PCOS, either when compared to the control population or after adjusting for body mass index values (156). These findings have been confirmed by a more recent study investigating the association between CV diseases and 10-year mortality in postmenopausal women with clinical features of PCOS, showing no association between clinical features of PCOS and mortality for CV diseases (157).

To sum up, there is evidence that life-long metabolic dysfunction in women with PCOS may exaggerate the CV disease risk, especially after menopause. Although all surrogate markers of CV risk are very common in PCOS, their association with CV events in PCOS still remains unclear. Therefore, uncertainty still exists as to whether PCOS status *per se* may increase CV mortality in women. Multicenter prospective studies in this area are warranted, possibly including confounding factors such as ethnicity, obesity and adequate evaluation of potential genetic substrates.

Another important area of androgen excess in women is represented by both CCAH as well as NCCA. In women with NCCA, androgen excess may persist with advanced age although very few prospective studies are available in this area. By contrast, patients with the CCAH are hyperandrogenic but at the same time they have an adrenal dysfunction which is characterized, from birth onward, by isolated cortisol deficiency either alone or associated with aldosterone deficiency, both requiring an adequate replacement therapy that can last a lifetime. So, it is mandatory to assess whether any metabolic

or CV disease that may occur over time may depend on the disease by itself or on the long-term hormonal replacement therapy. Unfortunately, few studies have been reported in this important area. The Swedish population-based national cohort study investigated the potential association of the CCAH with excess CV and metabolic morbidity. The study found that obesity, T2D, hypertension and hypothyroidism, but not CV diseases (including stroke, acute coronary syndrome, heart failure, aortic valve disease and obstructive sleep apnea) were significantly more frequent in CCAH patients ($n=335$) than in the controls ($n=33\,500$) (158). Another study (159) confirmed that 24-h systolic ($P=0.019$) and diastolic ($P<0.001$) blood pressure was significantly elevated in patients with CCAH compared to the controls. Finally, a more recent study found that, among 588 patients with CCAH having a well-defined mutation identified ($n=588$), the mortality rate for CV disease was not significantly different with respect to that found in a very large control population ($n=58\,800$) (160). Therefore, available studies do not confirm a high prevalence of CV events in adult or older patients with CCAH. Rather, current evidence seems to support the concept that, among potential factors for CV risk, androgen excess or, more likely, high doses of glucocorticoid replacement therapy may play a role.

In conclusion, both prevalence and incidence of the metabolic syndrome and T2D are significantly higher in women with PCOS than in the general population. By contrast there is conflicting evidence that with increasing age or after the menopause women with hyperandrogenic disorders, such as PCOS, may have an increased risk for CVDs or mortality. Intriguingly, in aged women with CCAH there is some evidence that the potential higher prevalence of metabolic and CV risk factors may be related to treatment with glucocorticoids and mineralocorticoids, rather than to the disease *per se*, although no prospective studies are as yet available in this area.

Aging male – late-onset hypogonadism

Although aging-associated changes in male gonadal axis may not be so dramatic compared to female, testosterone levels follow a downward trend during aging. Unlike women, there is no universally recognized syndrome ‘andropause’ and no specific age at which this process starts. It is rather an insidious process, mainly attributed to downregulation of hypothalamic-pituitary axis that eventually causes not only testosterone deficiency, but also a variety of clinical consequences (4).

Late-onset hypogonadism

Late-onset hypogonadism (LOH) is defined as a clinical and biochemical syndrome associated with advancing age and characterized by both symptoms/signs of hypogonadism and deficiency in serum testosterone (T) concentrations, below the reference range for the young healthy adult male (161). Diabetes, obesity and aging share an increased risk for LOH, through a vicious cycle. Testosterone (T) deficiency may predict or contribute to the development of insulin resistance and metabolic syndrome, whereas states of hyperinsulinemia/insulin resistance and obesity lead to a reduction of testicular T production with detrimental effects on the muscle and adipose tissue function (162).

Although in the majority of aging males serum concentrations of T remain within the reference range for young males, some aging males are characterized by mild symptoms of T deficiency (163). According to data from the European Male Ageing Study (EMAS), the prevalence of LOH is 2–3%, approximately (164). In addition, EMAS provided evidence of an association between LOH and overweight/obesity: LOH was diagnosed in 0.4% of males with body mass index (BMI) of $<25\text{ kg/m}^2$, 1.6% of males with BMI of $25\text{--}30\text{ kg/m}^2$ and 5.2% of males with BMI $>30\text{ kg/m}^2$.

Additionally, according to the existing data T deficiency appears to be common in type 2 diabetes. A large community-based cross-sectional study reported rates of subnormal T and free T levels in 29% of type 2 DM (165), while another study found subnormal calculated free T and T levels in 50% and 17% respectively (166). Men with type 2 DM have also been found to have a high prevalence of symptoms suggestive of hypogonadism such as fatigability and erectile dysfunction. However, the exact prevalence of LOH in diabetes, has not been clear due to different measures used in the various studies, namely low testosterone (T) or free T levels, or the full syndrome consisting of low T levels and the relative clinical symptoms and signs (166).

Serum total T decreases after the age of 55 years by 1–2% per year. During the same period, sex hormone-binding globulin (SHBG) increases by 2–3% per year; therefore, free T decreases more than total T, approximately by 2–3% per year (167). This T deficiency provides the pathophysiological background for LOH. Hypogonadism in LOH seems to be of mixed type; it is neither hypergonadotropic primary testicular failure, characterized by high concentrations of luteinizing hormone (LH), nor hypogonadotropic (hypothalamic or

pituitary failure, characterized by inappropriately normal concentrations of LH). The presence of central obesity in males with LOH has been associated with lower LH concentrations, implicating a central effect of obesity on the hypothalamic–pituitary–testicular axis (168). In 25% of men with type 2 DM, the hypogonadism is characterized by low T levels in association with low or inappropriately normal LH and FSH (hypogonadotropic hypogonadism), having no association with diabetes duration or HbA1c. In 4% of men with type 2 DM, subnormal T levels were associated with elevated LH and FSH concentrations (hypergonadotropic hypogonadism) (166). Several mechanisms account for the pathogenesis of LOH in DM, including hypothalamic kisspeptin changes, increased estradiol generation from the aromatase mediated T conversion in the adipose tissue, inflammatory cytokines, adipokines and insulin resistance (169, 170, 171). In Fig. 2 the differential rate of testosterone decline overtime between LOH and normal men is depicted.

According to its definition, the diagnosis of LOH requires (a) the presence of symptoms and signs of hypogonadism and (b) confirmation of hypogonadism through low serum T concentrations. According to EMAS (172), total T concentrations $<8\text{ nmol/L}$ (230 ng/dL) confirms the diagnosis of LOH; if total T is between 8 and 11 nmol/L ($230\text{--}320\text{ ng/dL}$), free T $<220\text{ pmol/L}$ (63.5 pg/mL) supports the diagnosis. A novel diagnostic entity is that of compensated hypogonadism, defined as low normal T ($>10.4\text{ nmol/L}$ – 300 ng/dL) and elevated LH concentrations ($>9.4\text{ IU/L}$) (173). Proper classification of LOH and exclusion of other causes of hypogonadism in the aging male is established through history, clinical evaluation (digital rectal examination) and additional laboratory (hematocrit (Ht), prostate-specific antigen (PSA), follicle-stimulating hormone (FSH), prolactin, SHBG, ferritin and drug screen) and imaging studies (pituitary MRI, bone densitometry), as needed.

In the interpretation of T levels, attention should be paid in conditions or medications that suppress T, such as acute reversible illness, opioid analgesia, glucocorticoids, statins and anti-diabetic drugs (e.g. pioglitazone and metformin) (174, 175, 176). T should be measured in the morning as it is subjected to marked diurnal variation, following an overnight fast (177). In borderline cases ($8\text{--}11\text{ nmol/L}$) it should be repeated in parallel with albumin and SHBG in order to calculate the ‘free T’ (172). Considerable controversy exists with respect to the optimal laboratory method for the measurement of T. The Endocrine Society suggests that full anterior pituitary

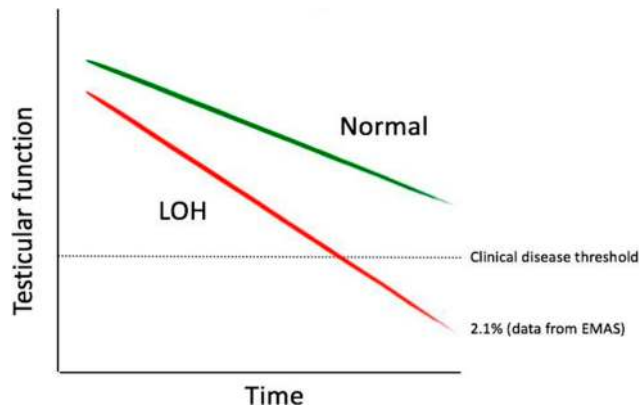


Figure 2

The differential rate of testosterone decline overtime between LOH and normal men.

function tests and imaging should be limited to those men presenting with T levels below 5.2 nmol/L (178).

The most prevalent symptoms of LOH involve sexual function: absence of morning erections, erectile dysfunction and loss of libido. Additional symptoms can be classified as physical (less vigorous activity, loss of capability of walking >1 km, reduced ability to bend and kneel) and psychological (feeling of sadness or downheartedness, loss of energy and fatigue). The clinical background of this picture includes muscle weakness and frailty, obesity, osteoporosis and depression (172). LOH has been associated with metabolic changes, including abdominal obesity, metabolic syndrome (e.g., type 2 diabetes mellitus), cardiovascular disease and chronic obstructive pulmonary disease. Lower levels of T are associated with an adverse lipid profile, namely elevated levels of triglyceride and LDL and decreased HDL levels, increased risk for cardiovascular disease and all-cause mortality (179, 180, 181).

Regarding therapy, the fact that males with LOH are characterized by T deficiency does not necessarily mean that T replacement in these males will result in an improvement of their clinical picture. In addition, the long-term safety of T replacement in aging males has not been established. There is need for randomized-controlled trials of superior methodological quality in order to establish the role of T replacement in the treatment of LOH. In any case, lifestyle modification, weight reduction and appropriate management of comorbid diseases constitute a prudent approach. Absolute contraindications for T replacement therapy include hormone-dependent malignancies, such as prostate and breast cancer; and relative contraindications include high

PSA concentrations (>4 ng/mL), erythrocytosis (Ht >50%), severe lower urinary tract symptoms (International Prostate Symptom Score (IPSS) >19), uncontrolled heart failure and untreated sleep apnea (178). Age *per se* is not a contraindication. T replacement therapy is expected to improve sexual function (especially in males with more severe hypogonadism) (182) as well as body composition (178). The effect of T on physical function, bone mineral density and metabolic syndrome are rather beneficial (183).

Concluding, LOH is not as prevalent as is thought to be, as symptoms/signs of hypogonadism and low T concentrations must co-exist for its diagnosis to be set. Nevertheless, it has been associated with increased morbidity and mortality, especially in overweight/obese males. Therefore, proper management with lifestyle modifications and T replacement in selected populations seems a logical approach; the latter has to be confirmed by prospective, interventional studies. Low T levels or LOH are common in patients with type 2 DM. T levels should be measured in all diabetic males while the T replacement should be individualized based on a careful evaluation of the risk-benefit ratio. More data are needed from large, well-controlled, randomized trials to help on the establishment of guidelines on the evaluation and treatment of diabetic males with low T levels or LOH, comparing the effectiveness of lifestyle measures and/or insulin sensitizing agents and the superiority of T replacement.

Aging beta cells

Across lifespan, the incidence and prevalence of type 2 DM increases considerably (184). In the US population almost one third of the elderly has diabetes, following an upward trend in comparison with past decades (185). The underlying mechanism(s) behind why DM is increasing in the elderly is still not clearly understood. It was initially attributed to the fact that older individuals display increased insulin resistance, increased adiposity, lower lean body mass and reduced physical activity. However, it has been shown that these factors alone do not account for age-related glucose intolerance (186). It is now widely accepted that intra-beta cell age-related dysfunction lies in the pathophysiological core of DM.

The decrease of islet cell function appears to be multifactorial and various theories have been proposed for understanding this age-associated dysfunction deeper. Firstly, it was shown that a reduction in glucose oxidation

rates and abnormal function of ion channels in beta cells with aging would result in a decreased insulin secretory response to glucose during old age (187). Simultaneously, the loss of expression of β -adrenergic receptors and orphan G-protein-coupled receptors encoded by GPR39, with a consequent reduction in adrenergic stimulation of insulin secretion and pancreatic function may also contribute to pancreatic dysfunction (4).

Beta cells display decreased regenerative capacity across aging, FoxM1, which regulates genes involved in cell cycle regulation and cell division is highly expressed in proliferating cells, and its expression decline in most cell types with age, including pancreatic islets (188). Beta cell proliferation is also reduced in humans with age. A small study of pancreata from twenty non-diabetic organ donors aged 7–66 years showed a decline in beta cell replication with age. The decline in beta cell replication was directly associated with a decrease in expression of the pancreatic and duodenal homeobox 1 (pdx1), a transcription factor, known to be important for beta cell replication (189). Indeed, there appears to be an impaired cell cycle entry of islet cells, which ultimately leads to decreased pancreatic mass (190).

Oxidative stress also plays a significant role in beta cell failure during aging. Oxidative stress can significantly compromise β cell function, as pancreatic β cells are innately more sensitive to oxidative stress. In an experiment by Maechler *et al.*, β -cells exposed to hydrogen peroxide activated the production of p21 cyclin-dependent kinase inhibitor and decreased insulin mRNA, ATP and calcium flux reductions in mitochondria and cytosol (191). Furthermore, as shown by Tiedge *et al.*, β -cells are lower in anti-oxidant enzymes levels (superoxide dismutase, catalase and glutathione peroxidase) and are more sensitive to ROS adverse actions (16). ROS affect beta cell function differently according to the levels of ROS and duration of exposure. Short-term exposure to low concentrations of hyperoxide derived from glucose metabolism is an important metabolic signal to elicit glucose-stimulated secretion of insulin. Although short-term exposure of β -cells to ROS might be beneficial in terms of promoting insulin secretion induced by glucose, chronic production of ROS and levels of ROS above a critical threshold might lead to β -cell dysfunction and/or death and reduction of insulin secretion (19). Furthermore, ROS production can also lead to the injury of β -cells through destruction of lipids in the cell membrane and cleavage of DNA (192). All the above, in conjunction with reserved proliferative activity of aging beta cell lead unavoidably to impaired insulin secretion and DM.

Anti-aging measures: to treat or not to treat

Diet

Nutrition is a crucial mediator of oxidative stress, contributing significantly to a plethora of metabolic disturbances. Specifically, nutrition-mediated oxidative stress, acting via multiple molecular pathways, promotes insulin resistance, beta cell dysfunction and eventually DM (90). This is perfectly depicted in the case of caloric restriction, which has been shown to delay aging and extend lifespan, through modulating multiple underlying mechanism of aging, including oxidative stress (1). Recent studies with mice and humans subjected to few days of fasting during a week have shown rejuvenation in the endocrine, immune and nervous systems of mice and improvement of biomarkers of diseases (DM, CVD and cancer) and regeneration in humans, without major adverse effects (193).

Additionally, modern, westernized dietary habits, with an increased consumption of carbohydrates and fat and decreased intake of dietary fiber, can predispose aging individuals to metabolic disturbances, through modulating the composition of gut microbiota (194). The intestinal mucosa represents the biggest mucosal surface of the body and it is in direct contact to the gut microbiota, several toxins and many allergens from the diet. It has been demonstrated that altered intestinal microbiota leads to increased intestinal permeability and deregulated mucosal immune response and has an important role to play in both inflammatory and allergic diseases (195). Aging *per se* also has a major impact on chronic diseases as the natural changes occurring during aging, such as decreased gastric motility and secretion, changes in dietary intake and frequent use of several medications may all promote chronic inflammation due to a deregulation and lack of richness of gut microbiota, promoting the presentation of chronic inflammatory diseases, including obesity, insulin resistance, diabetes and other metabolic disturbances (196).

Anti-oxidants

Anti-oxidant supplementation could potentially act favorably on preserving beta cell function and delaying the onset of metabolic disturbances. In fact, in various experimental models anti-oxidant supplementation was shown to protect pancreatic cells from glucotoxicity and lipotoxicity, through reducing apoptosis rates, improving insulin gene expression and insulin secretion (197, 198). However, more studies are necessary to evaluate whether

anti-oxidants have a clinically significant favorable effect in pancreatic function in humans.

Insulin agents

Beta cells insulin receptors are indeed acting by favoring glucose entrance into the beta cells so to amplify the insulin secreting response. *In vivo* experiments by insulin clamp have shown that this mechanism is able to increase human insulin secretion by about 40% (199). As long as insulinemia is only based on the pancreatic secretion this mechanism allows a stronger reaction to hyperglycemia without counter-effects: indeed, as soon as the amount of glucose into the beta cells raises too much, the parallel increase of the $\cdot\text{OH}/\text{H}_2\text{O}_2$ causes inhibition of the autocrine insulin receptors and stops insulin self-induction avoiding the occurrence of hypoglycemia. If rather hyperinsulinemia is sustained by therapeutic administration, therapeutic insulin doses will continue to stimulate the beta cells insulin receptors with the potential to overcome the regulatory blockade and to further sustain glucose entrance, insulin release and $\cdot\text{OH}$ generation. Thus, therapeutic insulin in subjects whose beta cells are under mitochondrial dysfunction has the potential to exacerbate the risk to accelerate beta cells degeneration.

The negative outcomes resulting from an intensified glycemic control reported in the ACCORD trial (200), i.e. increased mortality among patients undergoing intensive treatment, might be to a good extent explained by the above mechanism. Thus, it is worth to look at the opposite intervention strategy, i.e. to restore the ability of the cell to manage the energy production and to consume glucose without suffering from excess $\cdot\text{OH}$ leakage. This can be achieved of course by avoiding high fat, high glucose meals but also by restoring and/or strengthening the reactivity of the natural anti-oxidant system and the availability of intracellular and mitochondrial glutathione (GSH).

Furthermore, insulin regimens have been shown to display a respectable variability of insulin absorption between subjects or interindividual variability, as well as within subjects or intraindividual variability (201, 202). This in turn affects the efficacy of insulin therapy, resulting in suboptimal metabolic control, either through hyperglycemia or hypoglycemia, which both have a negative impact in beta cell function. The extent of insulin absorption variability is related to the pharmacokinetic properties of each insulin preparation. Exogenous insulin must be in the form of monomers

and dimers to be able to diffuse in the interstitial fluid and enter the blood vessels. Therefore, the rhythm and extend of insulin hexamers formation at the injection site in subcutaneous adipose tissue and the dissociation of insulin hexamers to monomers are important regulators of exogenous insulin absorption rate (203). Another important factor affecting insulin absorption is the resuspension of insulin preparation, which is mandatory before each injection for all crystalline suspensions of protaminated insulins. This applies not only to NPH human insulin and biphasic premixed human insulin preparations which contain NPH insulin, but also to biphasic premixed insulin analogs as well (204). Several factors related to the injection technique may also affect insulin absorption. Injection area (anatomic region), insulin injection depth and lipodystrophy reactions can alter insulin pharmacokinetics and glucose homeostasis (202, 205). Additionally, factors affecting subcutaneous blood flow have a significant impact on insulin absorption. Increased skin temperature of the injection site leading to vasodilation accelerates insulin absorption, e.g. physical activity (206). Finally, medication errors, resulting from wrong assessment of patient's needs by the doctor or inadequate patient education by the pharmacist, should be avoided. Thus, clinicians dealing with patients with insulin-treated DM should consider the issue of insulin absorption variability and try to minimize it, in order to achieve optimal metabolic control and preserve their remaining beta cell activity.

Conclusion

Endocrine system undergoes major alterations during aging, affecting the majority of body function. Many of these changes have been so far well described, but their exact impact on health and disease remains unknown. Furthermore, it is important to differentiate, which of these aging-associated alterations constitute beneficial, physiological adjustments of human body to environmental stimuli and to the aging process itself and which alterations are actually harmful to cell viability. This would further help us develop targeted medical interventions, with long-term hormonal replacement/blockade with one or more hormones that would promote longevity. As life expectancy increases, it is important not to accept the brutal stereotype that aging is unavoidable, delineate the pathophysiology of aging and take advantage of longevity pathways in order to allow us live not only longer but also with the best quality of life.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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