

Chapter 4

Sex Differences in Aging and Associated Biomarkers



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1 Introduction

Aging is a natural process defined by the gradual, time-dependent decline of physiological and behavioural function, and individuals of the same chronological age can show great variability in these parameters [1]. The capacity of biological systems to continuously adjust for optimal functioning despite ever changing environments is essential for healthy aging, and variability in these adaptive homeostatic mechanisms may reflect the heterogeneity observed [2].

Adaptive homeostasis refers to “The transient expansion or contraction of the homeostatic range in response to exposure to sub-toxic, non-damaging, signalling molecules or events, or the removal or cessation of such molecules or events” [3], and represents the framework in which the body dynamically maintains biological and behavioural processes required for life. The endocrine, metabolic and immune systems are well established in the literature as homeostatic mechanisms [3]. These systems are complex and integrated, involving feedback-dependent mechanisms and cross-talk across networks, thereby contributing to overall system regulation [4]. These integrated systems are ultimately governed by the complex interaction between genetic and environmental factors, including lifestyle factors, which may affect homeostatic mechanisms and ultimately change the progression of aging. Growing evidence suggests that the adaptability of these homeostatic systems decline with age, with many linked to age-related morbidities suggesting a shared pathophysiology of disrupted cellular homeostasis [2]. This change or decline in key homeostatic systems may therefore reflect the inability to activate or modulate several adaptive responses.

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With an ever-increasing aging population, interest in biomarkers of healthy or accelerated aging is growing. Although there is no universally accepted definition of ‘aging’ biomarkers, attempts to identify objective biomarkers often point to mediators of homeostatic systems including physiological functions, cognitive processes, and endocrine and immune functions [5]. Importantly, sex differences are often reported in many of these adaptive systems, and this may reflect, to some extent, sex differences observed in aging and age-related disease states. Therefore, it is imperative to consider sex and gender differences in aging and age-related diseases.

Known sex differences in key homeostatic mediators may influence the differential susceptibility and resilience to aging outcomes between males and females. Therefore, within the context of biomarkers in aging, it is important to consider biological sex differences and the role of gender, which includes a person’s psychosocial and cultural self-identification [6], and how these may influence analytical measurement. From a physiological standpoint, biological sex may play an important factor in the variability of biomarkers due to the capacity of sex chromosomes and sex hormones (i.e., estrogen, testosterone), to alter biomarkers present in blood, saliva, cerebral spinal fluid, or tissue samples [4]. From a behavioural standpoint, biological sex hormones, sex chromosomes, and gender may impart differential adaptive processes seen in the cognitive domain [7].

This chapter aims to outline sex differences in key homeostatic domains thought to be associated with the pathophysiology of aging, and which are often proposed as biomarkers of aging and age-related disease states. This includes summarising the available literature of sex-based differences and hormonal status with regards to the gonadal and adrenal endocrine systems and immune function.

2 Sex Differences in Age-Related Diseases

Sex differences in longevity, mortality and age-related diseases are well established in the literature. In developed nations, females tend to live longer than males and have notably lower death rates than men at all ages. However, it is also known that females suffer from higher levels of morbidity than men [8]. Salient gender-related differences have been associated with risk, clinical expression, treatment response and course of several age-related neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease [9].

Alzheimer’s disease, the most common cause of dementia, disproportionately affects females, with a higher incidence compared to males [10]. Females have a faster rate of cognitive and functional decline following diagnosis and appear to suffer significantly greater cognitive impairments after accounting for sex differences in age, education and dementia severity [11]. Sexual dimorphisms in Alzheimer’s disease pathologies have been reported in several mouse models, with females expressing greater pathology [12]. Sex has also been demonstrated to modify genetic risk factors for Alzheimer’s disease. For example, the apolipoprotein E gene $\epsilon 4$ allele confers a greater Alzheimer’s disease risk in females, as compared to

males [13]. In contrast, males have at least a two-fold greater risk and prevalence of Parkinson's disease [14]. In comparison to females, males have a later onset of Parkinson's disease. Clinical and cognitive profiles also differ between males and females with Parkinson's disease. Males are more likely to present with rigidity, rapid eye movement behaviour disorder and sleep disturbances. The cognitive profile in males is more likely to include verbal fluency deficits and a lack of facial emotions. Females are more likely to present with a tremor dominant form of Parkinson's disease. A reduction in visuospatial cognition is more frequent in females [9, 15, 16]. Possible reasons for these sex differences in age-related, neurodegenerative disorders and longevity are thought to be due to intrinsic differences based on genes, sex hormones, and reproductive physiology. These can confer differential risks of morbidity. In addition, extrinsic factors such as lifestyle, health habits, exercise, nutrition, and others may also have a connection with sex difference in biological vigor as potential moderators.

3 Endocrine Markers of Aging

The endocrine system represents a key facilitator of homeostatic function, maintaining critical functions including development, growth, metabolism, stress responses, reproduction, and sleep patterns. As such, it is one of the most important regulators of physiology over the lifespan. The hypothalamus can be considered as the link between the nervous system and endocrine system, continually adjusting according to internal and external environments using feedback mechanisms, and subsequently regulating all hormone-related physiology and behaviour. With advancing age, alterations in hormonal networks, with concomitant excesses or deficits in steroidal hormones occur [17]. This is further exacerbated by poor sensitivity of tissues to their action, and a decreased sensitivity toward feedback control [18]. These alterations are thought to be translated clinically to losses of function seen in aging of the reproduction system including menopause, developmental and maintenance growth axes, and the adrenal endocrine axis [18].

4 Aging of the Female and Male Reproductive System and the Aging Gonadotropic Axis

Sex steroid hormones, including estrogens and androgens (testosterones) are regulated by the hypothalamus pituitary gonadal (HPG) axis. While the endocrine feedback mechanisms are complex, a simplified account of the HPG axis suggests that the hypothalamus releases gonadotropin-releasing hormone (GnRH), stimulating the anterior pituitary to produce and secrete gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The ovary, comprised of two cellular

components, is synergistically stimulated by FSH and LH, leading to the release of sex steroids [19]. FSH and LH then complete the endocrine feedback loop by posing both positive and negative feedback effects on the axes, depending on timing and context [20].

A clear change observed throughout aging is the decline in sex hormone production. As women age, the natural transition from reproductive years to menopause is associated with fluctuating and eventual decreasing levels of ovarian sex steroids. Suggested to be a predictor of reduced ovarian reserve, gonadotropin FSH increases pre-menopausally [21]. Subsequent to the menopausal transition, eventual decreases in FSH and LH occur [18]. Again, alteration at the level of the hypothalamus-pituitary-gonadal axis is thought to principally contribute to this progressive decline. This appears to be moderated by increased levels of GnRH which increase with age with hypothalamic adaptations during aging, favouring its release [22, 23]. Moreover, the feedback effect of estrogen on FSH responsiveness is greater than on LH and is attenuated with aging [24].

This natural consequence of aging of the reproductive axis and sex-steroid privation leads to the loss of reproductive capability, in addition to a sequelae of clinical symptomatology including loss of muscle and bone mass, visceral tissue accumulation, insulin resistance, and negative impacts on mood, cognition, and importantly, quality of life [25]. A recent meta-analysis of 310,329 women demonstrated that younger age at menopause (occurring prior to 45 years old) was associated with a higher risk of coronary heart disease, stroke, cardiovascular mortality, and of all-cause mortality [26]. In addition, the occurrence of age-related morbidities including Alzheimer's disease and Parkinson's disease has led to the suggestion that the loss of sex steroid hormones, in both female and males, is a contributor to the aetiology [27, 28].

Although not as dramatic as seen during the female menopausal transition, a modest, progressive decline in anabolic androgen is observed in males throughout the aging process [29]. No specific age can be defined as to when this process begins, although several cross-sectional and longitudinal studies have confirmed that testosterone (free and bound) decline in aging, while LH increases with age [30–34]. Recently, weight and lifestyle factors have been shown to moderate these hormonal changes [30]. In addition, the levels of sex hormone binding globulin (SHBG) increase during aging which binds testosterone, resulting in relatively less free biologically-available testosterone [35]. Clinically, low testosterone levels have been associated with increased mortality in men, showing an approximate doubling in mortality risk compared to men with normal levels [36]. Associations between low testosterone and adverse outcomes have also been reported, including diabetes mellitus, osteoporosis, decreased libido, and cardiovascular disease [37–39]. In a recent biomarker analysis that included sex hormones in an aging 'panel', it was reported that low levels of testosterone are correlated with high-sensitivity cardiovascular risk factors, consistent with a potential increased risk of cardiovascular disease [40].

Fluctuating hormone levels throughout the menstrual cycle of reproductive aged women and in perimenopausal women should be also be considered, as female hormonal status has been shown to modulate many age-related biomarkers. As an

example, stress-induced cortisol levels are associated with impaired emotional retrieval [41] and affect serum biomarkers in clinical tests only in some menstrual phases [42]. In addition, hormonal contraceptives used by many women of reproductive age have shown to vary clinical measurement of serum biomarkers [42], and this is rarely considered within the context of system dynamics and levels of molecular outputs. Although the contributions and mechanisms are yet to be fully elucidated, sex steroid hormones exhibit clear functional activity on age-related processes and may govern, in large part, the sexual dimorphisms observed in many age-related morbidities.

5 Dehydroepiandrosterone in Aging

Adrenal secretion of dehydroepiandrosterone (DHEA) also profoundly fluctuates across the lifespan and represents one of the largest endocrine changes observed in human aging. Although the physiological role of DHEA is not yet completely understood, its apparent influence in age and age-related disease states has yielded much interest.

DHEA is predominately synthesised within the adrenal zona reticularis of the adrenal cortex in response to adrenocorticotrophic hormone (ACTH) and subsequently released into the bloodstream [43]. The majority of circulating DHEA is converted to its sulphated derivative DHEA-S via the enzyme steroid sulphotransferase, which is mostly bound to the carrier protein, albumin. As the precursor to the sex steroid hormones, it is also synthesised to a smaller extent in human gonads [44]. DHEA-S serves as a precursor to approximately 50% of androgens in adult men, 75% of active estrogens in premenopausal and nearly 100% in postmenopausal women [45]. More recently, reports pertaining to its *de novo* synthesis in human brain highlights the important neurosteroidal properties that DHEA may have, posited to have direct activity at estradiol receptors [46] and NMDA receptors [47], possess neuroprotective properties, and participate in neurogenesis, catecholamine synthesis and secretion, in addition to anti-inflammatory effects [44]. Indeed, it has been reported that concentrations of DHEA/DHEA-S are considerably higher in the brain than in any other organ.

At birth, DHEA-S levels are markedly high until involution of the adrenal fetal zone, which leads to a cessation in DHEA production and it remains dormant until the childhood period, when production once again commences. Peak levels for both females and males are observed when they are in their thirties, after which point DHEA levels fall by approximately 2–3% each year [48], declining to levels that are approximately 20% of peak values in men and 30% of those in women [43, 49].

Several studies have reported the association between low levels of DHEA-S and DHEA and frailty in older populations [50–52]. In addition, several cross-sectional studies, including investigations of serum and cerebrospinal fluid (CSF), have implicated DHEA levels in a number of age-related pathologies including type 2 diabetes mellitus, psychiatric disease states, and neurocognitive disorders, including

Alzheimer's disease [29, 43]. Although many studies have provided support for a positive correlation between higher levels of DHEA and improved health outcomes, including muscle strength, higher bone density and increased longevity in males, other studies have demonstrated that DHEA is associated with negative health outcomes. For example, lower DHEA-S levels have been associated with greater risk of ischemic stroke, having been adjusted for appropriate potential confounders [53].

Due to such accumulating literature, DHEA has drawn much attention as a putative 'anti-aging' supplement, although little is known about the underlying mechanism with regards to its progressive decline and associated pathologies. Hypotheses pertaining to plausible biological mechanisms include the ability of DHEA/DHEA-S to modulate energy metabolism, systemic inflammation, and that it may counteract or modulate the stress endocrine hormone, cortisol [54–56]. Given the critical role of sex steroids and age-related associations, the function of DHEA as an alternative source of androgens and estrogens to local tissues may be a plausible way in which DHEA/DHEA-S protects against aging and age-related pathologies [57]. Although there is some evidence to suggest that DHEA supplementation may improve skin status and bone turnover in older females, there are few clinical studies powered enough to conclude the effects of DHEA on aging and age-related diseases. In addition, data on the safety profile of long-term DHEA supplementation are still lacking [58].

5.1 Sex Differences in DHEA

Differential trajectories of DHEA levels between the sexes begin following the onset of adrenarche, and differences in levels can again be observed during the perimenopausal period in women, and in older people [59]. Peak levels have also been reported to occur earlier for females [60]. The biological reason for this difference has yet to be elucidated but it has been suggested that higher levels observed in males may reflect chromosomal differences [61]. The gene for steroid sulfatase, which degrades androgens, is located on the X chromosome. Therefore, as males only have one copy of the gene, they consequently have less degradation and higher DHEA concentrations [57]. Although the literature reports somewhat inconsistent results, a recent review concluded that enough evidence supports the association between DHEA and aging in males, but in females this may not exist, or displays a non-linear, U-shaped association. Larger studies, with a prior stratification of sex, are required to confirm these findings [62].

6 Analyses of Steroidal Hormones

The complexity of sex steroid measurement should not be underestimated, and there is current debate over the best technologies to use (see [63] for overview). These steroids exhibit only modest differences in structure, but which amount to profound

functional differences. Owing to such subtle differences in structure and small size, steroids are not easy to measure.

Although immunoassays have remained the mainstay of steroid analysis, the reliability of the specificity and sensitivity of these assays have come into questions, particularly commonly used commercially derived assays [64]. Unless preanalytical extraction methods or chromatographic steps are introduced to purify samples prior to the immune-assay, these assays are prone to inaccuracy due to their non-specificity [63]. Mass spectrometry has introduced far greater specificity of steroidal hormones and allows for specific analysis of different steroids (i.e., estrogen, and testosterone). Currently this technique provides the highest specificity due to mass spectrometry providing the exact structural information of the analyte, allowing for specificity between steroid analytes and their precursors. However, it is noted that costs and expertise required for such analyses may prevent many researchers from conducting such analyses. As technology advances and becomes cheaper, this gold standard technology will grow in use.

Relevant to this chapter, difficulty in the measurement of steroids has been proposed for the reason for the inconsistent findings seen in the DHEA aging literature. The low specificity and precision of immunoassays may have yielded incorrect measurements in subjects low in DHEA/ DHEA-S [62].

7 Aging of the Adrenal Endocrine System: The Hypothalamus-Pituitary-Adrenal Axis

Activation of the hypothalamus-pituitary- adrenal (HPA) endocrine axis and the subsequent stress response is a fundamental homeostatic mechanism that enables a wide array of physiological and behavioural responses to actual or perceived threat. Initiated at the level of the hypothalamus via corticotrophin releasing hormone (CRF), a signalling cascade then ensues with adrenocorticotropin hormone (ACTH) released from the anterior pituitary, followed by glucocorticoids being synthesised from cholesterol in the adrenal cortex, and released directly into the bloodstream. Glucocorticoids are lipophilic and largely transported bound to cortisol binding globulin (CBG) leaving only a small fraction (10%) biologically active [65]. As glucocorticoids can readily cross the blood-brain-barrier, the steroids consequently can exert action at both local central nervous system level and systemic peripheral levels, where it induces or represses the transcription of a plethora of target genes [66, 67]. In this way, glucocorticoids orchestrate a vast repertoire of adjustments to adapt to external or internal changes to the environment, ensuring optimal regulation of several interlinked regulatory systems including reproduction, growth, and immunity, and cognitive signalling processes [68]. In addition, fast acting, non-genomic action of glucocorticoids occur, engaging glutamatergic system alterations [69].

Glucocorticoids mediate its own activity and termination via two receptors; the high affinity type 1 mineralocorticoid receptor (MR) and lower affinity type 11 glucocorticoid receptors (GR) [70]. The high affinity of MR to glucocorticoids is thought to be responsible for regulating tonic activity and dictates the basal circadian and ultradian rhythms observed across the day [71]. GR are found widely distributed throughout the brain, with high density found in most neurons and glia. With lower affinity to physiological GC, these receptors only become functionally activated as the stress endocrine hormone cortisol rise following a stressor and play an important role in mediating GC effects on mobilising energy stores, inflammation and neural function [72]. Importantly, it is thought to be primarily responsible for blunting further activity of the HPA stress response. Both receptors act in synergy to mediate HPA dynamics important for several of these systems of homeostasis, eloquently fine tuning the HPA dynamics depending on the necessary action. An example of this is found within cognitive processes, such as learning and memory. GR and MR are both abundantly expressed in neurons of the hippocampus, prefrontal cortex, and amygdala [73] where they are thought to have opposing functions which regulate hippocampal synaptic neuroplasticity during the stress response, important in long term potentiation mechanism of learning and memory. Preclinical rat models have demonstrated that activation of the MRs may be a prerequisite for hippocampal plasticity, while GRs may exert an inhibitory effect of plasticity [74]. Although initial sensitivity to feedback of glucocorticoids due to the high expression of GRs and MRs, after repeated responses seen in acute and chronic stress, the hippocampus down-regulates receptor expression [75]. In line with this, although acute, moderated activation of the HPA axis is a necessary and beneficial response, such chronic activation may lead to tissue damage or receptor desensitisation, resulting in attenuated negative feedback, and ultimately adverse outcomes [76]. Individual differences in vulnerability and resistance to stress, governed by HPA dynamics [77] may thereby contribute to the heterogeneity of the aging, and age-related diseases.

There is evidence that HPA axis dynamics [78] and GR expression levels in areas of the dentate gyrus [79] change over the natural process of aging and are postulated to be a major driver in accelerated aging, with lower diurnal cortisol levels having been associated with longevity [80]. In healthy humans, the HPA axis, and glucocorticoid output typically follows a diurnal pattern. The typical pattern displays a sharp increase upon awakening, known as the cortisol awakening response (CAR), followed by reduced levels throughout the day, until the sleep nadir [81]. Increased basal cortisol output [82], diurnal amplitude flattening [83], and an altered CAR response both in magnitude and in day to day variability [84] have all been associated in aging models [61]. In addition, attenuated negative feedback by glucocorticoid receptors at the level of the paraventricular nucleus, hypothalamus, hippocampus, and prefrontal cortex have been reported [61, 70]. Furthermore, the 11- β hydroxysteroid dehydrogenase enzymatic activity responsible for the interconversion of inactive cortisone to active cortisol has been reported to decrease with age, resulting in the inefficient inactivation of active cortisol to inactive cortisone [85]. The two isoforms of this enzyme have also been suggested as a potential key

mechanism in the acceleration of aging [86]. In addition, the systemic 11- β hydroxysteroid dehydrogenase isoform 1 is predictive of progressive brain atrophy and cognitive decline [87].

Such age-related changes in HPA dynamics are often related with declined cognitive processes, cardiovascular alterations, and psychiatric disease states. For example, a higher 24 h urine output has been associated with Alzheimer's disease [88]. Mechanistically speaking, this may be a result of cortisol induced apoptosis and death of hippocampal neurons [89], as observed by decreased adult hippocampal neurogenesis in an Alzheimer's disease model [90].

7.1 Sex Differences in HPA Axis Regulation

Research suggests there are substantial sex differences in HPA-axis dynamics and cortisol levels during regular diurnal maintenance and in response to stress, which may contribute to differential aging dynamics between the sexes [91]. In vitro models have shown that female rodents exhibit greater basal corticosterone production by the adrenal glands [92] and have a more robust HPA axis response to both physical and psychological stressors, thought to be a result of circulating estradiol levels [93]. Clinical investigations have also provided support for the sexual dimorphism of the HPA axis stress response [94]. The HPA axis stress response in females is characterised by a larger, more sustained secretion of ACTH and cortisol, suggesting enhanced activity and reduced negative feedback [95], while the pulsatile cortisol feedback on ACTH mediated by GC receptors appears to be influenced by sex [96]. In addition, there is evidence indicating that both GRs and MRs are less sensitive to cortisol modulation in females than males, suggesting reduced feedback by autoregulation of these receptors [70].

Although the exact mechanism of action remains to be elucidated, estradiol has been shown to enhance HPA activity, while testosterone appears to have an inhibitory effect by acting upon the hypothalamus [97]. In contrast, there is also evidence to indicate that estradiol, but not testosterone, heightens the cortisol-mediated negative feedback on pulsatile ACTH secretion in both aged men and women [96]. Further research must be conducted to understand this complex interplay between the HPA and HPG axis, across the life-course and considering sexual dimorphisms.

In line with these findings, a clear association between menstrual cycle phase and cortisol basal measurement has been shown. Women in the luteal phase of the menstrual cycle have a similar cortisol response to men, whereas in the follicular phase, attenuation of cortisol production has been shown [98]. Notably, increased CBG is known to occur in cases of estrogen excess (e.g., pregnancy and use of estrogen-containing oral contraceptives, resulting in higher concentrations of total cortisol [99], although free cortisol levels are usually unaltered in states of increased CBG [100].

It is also now appreciated that prenatal exposure to early life stressors and increased levels of glucocorticoids can influence later life behaviour, biological processes, and susceptibility for disease states. Support for sex differences in such early-life programming of the HPA in humans has been demonstrated in rodent models, with evidence for the female HPA axis being more vulnerable to stress and glucocorticoid programming, and female offspring demonstrating an increased HPA reactivity [101]. This may be one mechanism underlying sex differences in later life diseases and sex dimorphisms shown in aging.

7.2 *Measurement of the HPA Axis*

Glucocorticoids have traditionally been assayed using a range of substrates including blood, saliva and urine that provide a sampling procedure that is simple and relatively non-invasive [102]. More recently, cortisol has been measured using hair sampling, which gives a chronic reading of cortisol levels [103]. Salivary cortisol represents the bioavailable, active form of the molecule that escapes the CBGs in the salivary glands and saliva [65]. In addition, unbound cortisol is excreted in the urine and 24 h measurements correlate well with those in serum in cases of cortisol excess [65]. Hair analysis provides the opportunity to determine past cortisol exposure and represents a retrospective index of cumulative cortisol output which is not influenced by the circadian rhythm of the HPA-axis, or by acute stress. As hair grows at an average rate of 1 cm/month, the 1 cm segment closest to the scalp represents total cortisol levels during the most recent month, with the outer segments representing previous months [103–105]. However, several limitations should be noted, as different hair care routines (e.g., use of hair dye), seasonal variations, and storage length have all been documented to influence readings [106]. The time of sample collection is also a critical consideration as cortisol follows a circadian rhythm of secretion. The cortisol awakening response (CAR) is measured using a minimum of three separate sampling times using blood or saliva (upon awakening, 30 min after waking, and 45 min after waking) [107]. However, the majority of studies employ a ‘minimal protocol’ by analysing one time point only, which has potential implications for the reliability of measurement. For cross-sectional studies it is recommended that up to six consecutive days of samples should be collected to accurately assess single time-points, and that four measures at two consecutive weeks days are required to reliably measure the CAR as a trait measure [108]. HPA axis dynamics and feedback sensitivity can also be measured using a synthetic glucocorticoid, dexamethasone. In clinical testing of suspected hypercortisolemia, the dexamethasone suppression test (DST) can detect abnormal HPA axis activity by a failure to reduce total cortisol production via stimulating the negative feedback mechanism and ACTH production. Interestingly, this has been found to be moderated by genetic variation [109] and use of hormonal contraceptives. The false positive effect found in fertile women using hormonal contraceptives, due to the resultant increased levels of CBG and consequent increase in total cortisol concentrations,

was found to be eliminated after one-week cessation of contraceptive use [110]. Various stressful stimuli that can reliably induce the cortisol response in human research participants can also be used to evaluate HPA dynamics. These include standardised psychosocial protocols, such as the Trier Social Stress Test (TSST), and physical stressors (electrical stimulation).

8 Aging of the Immune System

A functional immune system is critical for protecting the host against infections and malignancies, regulation of wound healing, and ultimately for separation of the 'self' from surrounding organisms that compete for space and resources [111]. The immune system is coordinated into two sub-classifications that govern the immune response. The innate immune system mounts a fast, non-discriminatory response, initiated by macrophages and dendritic cells that produce inflammatory mediators, including histamine, bradykinin, serotonin, leukotrienes, prostaglandins, cytokines and several growth factors and enzymes. This promotes either tissue repair/regeneration with subsequent resolution of inflammation, or tissue remodelling/fibrosis [112]. The adaptive response is initiated if the innate immune response fails to respond sufficiently, creating an immunological memory after the initial response to a specific pathogen. This more sophisticated arm of the immune system requires recruitment of B and T cells, followed by antibody and cell-mediated responses, respectively, and consequently has a targeted, albeit slower action [113].

Like all successful adaptive homeostatic responses, subsequent to the activation of the immune response, an ensuing cessation or resolution of inflammation must occur, returning the tissue environment to the basal state allowing for tissue repair/regeneration. Recruitment of macrophages to an anti-inflammatory phenotype is critical to this process and involves the release of anti-inflammatory cytokines including interleukin (IL)-10 and TGF- β , as well as lipid mediators [114]. An over-activated immune response leads to tissue remodelling (tissue fibrosis), autoimmune and neurodegenerative diseases, rather than tissue regeneration [114, 115]. Changes in inflammatory mediators have been identified in age-related syndromes including type 2 diabetes, Alzheimer's disease and cardiovascular disease [116].

During aging, the immune system loses the ability to protect against pathogens effectively, and fails to support appropriate wound healing [111]. Such a decline in effective immunological functioning results in these subjects not responding efficiently to novel or previously encountered antigens [117]. The adaptive immunological arm undergoes complex changes in the aging process that includes epigenetic and metabolic changes affecting naïve, memory and effector T cells, and B cells [118]. A decrease in the number of naïve T lymphocytes and increase in memory and effector T cells occurs with age, in addition to the reduced diversity in T-cell receptors with consequential diminished functionality of naïve and memory T-cells [116]. Clinically, this results in most vaccines being less immunogenic and efficient in the elderly [119].

Additionally, aging is also associated with progression toward a chronic, sub-clinical inflammatory state, characterised by elevation of peripheral pro-inflammatory chemical mediators in the absence of overt infection, a term coined ‘inflamm-aging’ [120]. Several human studies have demonstrated an association between elevated levels of cytokines, especially IL-6, and a decline in innate immune function in older individuals [121]. Such a peripheral inflammatory state has been associated with the susceptibility and progression of many age-related disease states including cancer, diabetes [122], and cognitive and structural brain changes [123]. Moreover, it has been demonstrated as a key risk factor of mortality [124]. In addition, the innate arm of the immune system initiates cross talk with the adaptive arm through antigen presentation, co-stimulatory molecule expression and cytokine production, and consequently can contribute to adaptive immune responses [125]. More recently, it has been discovered that at sites of infection, adaptive immune memory cells can also regulate the innate inflammatory response, by the epigenetic reprogramming of innate immune cells by previous experiences [126]. Interestingly, it has been hypothesised that centenarians exert a counter action to this pro-inflammatory state, by producing increased amounts of anti-inflammatory cytokine mediators including IL-10 and TGF- β [127].

8.1 Sex Differences the Immunological Response

There is a growing body of evidence demonstrating inherent sex differences in the immune response, which includes both the innate and adaptive arms of the immune system. On average, females exhibit a stronger innate and adaptive immune response than males, resulting in improved pathogen clearance, vaccine efficiency, but greater predisposition to autoimmunity and chronic inflammatory disorders [128]. Sex chromosomes contribute to genetic differences due to the fact that many immune-related genes (e.g., FoxP3 and toll like receptors 7 and 8) are present on the X chromosome. As the possibility of incomplete X-chromosome inactivation occurring in females exists, this can lead to over-expression in females of such immune related genes [129, 130]. Sex hormones can also interact with genetic and environmental factors that determine immunity and appear to influence multiple aspects of the immune system, including the contribution to cell differentiation, cytokine profiles, and epigenetic alterations [130, 131]. Estrogen receptors have been shown to regulate cells and pathways in both arms of the immune system in a predominately stimulatory fashion, in addition to immune cell development [132]. On the other hand, androgens have been shown to be primarily immunosuppressive, suppressing immune reactivity and inflammation [133]. Evidence for sex disparity does exist in the immunological response throughout aging, although the majority of studies do not stratify for sex. Females who are in the menopausal period of their reproductive lives show increased peripheral pro-inflammatory markers, higher numbers of natural killer immune cells with reduced cytotoxicity, and reduced numbers of B and T

cells, relative to premenopausal females [134]. Research investigating differential sex influence in the innate immune system is still in its infancy, although data exist suggesting an elevated production of inflammatory cytokines and proteins in females compared to males persists among aged individuals [135, 136]. With regards to adaptive immunity, proliferation of T cells, central to the adaptive immune response, shows a decrease with aging, although this rate of decline was significantly lower in females, than in males [137]. Sex differences have also been noted to occur in response to vaccines protecting against influenza, tetanus, pertussis, shingles and pneumococcal infections, and the efficacy of vaccines in older individuals is consistently higher in females than in males [136].

Collectively, the major changes of the innate and adaptive immune system with aging result in vulnerability to certain infections, and decreased efficacy of many vaccines. Although sex differences in the aging immune system have not been studied extensively, addressing such sex differences may aid in greater effectiveness of vaccines and immunotherapies [130]. As several diseases associated with age are also sensitive to alterations of the immune system, it is important to evaluate potentially-associated sex differences in the underlying pathophysiological processes.

9 Conclusions

The capacity of homeostatic biological systems to continuously adjust for optimal functioning during healthy aging may reflect heterogeneity observed in the aging process. Importantly, such systems including endocrine, immune and cognitive systems appear to be moderated by sex, which may be a defining factor in determining sex-based differences in age-related processes, and diseases. Aging appears to be multi-factorial and characterised by the dysregulation of complex system dynamics, namely the interplay of these homeostatic mechanisms driving the aging process. It can be hypothesised that dysregulation in any of these systems may impose feed-forward alterations on other systems, and dysregulation early in the accelerated aging process may be different for different people. This makes it particularly challenging to research this field, especially early in the aging process. Composite biomarker panels are required at multiple time points in order to target multiple systems and to better understand the chronicity of biomarkers during aging. Moreover, it is critical that future studies specifically address sex-effects when studying the aging process and age-related diseases, to enable sex differences, which are non-modifiable risk factors, to be differentiated from potentially modifiable lifestyle and environmental risk factors. This is crucial to advance therapeutic options that may include sex-based and hormone-based interventions. Policies put forward by the National Institute of Health regarding the requirement of sex and gender inclusion plans in both preclinical and clinical research, underscores this point. Biomarkers of aging cannot escape this necessity.

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