Oxidative stress and the ageing endocrine system

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Abstract | Ageing is a process characterized by a progressive decline in cellular function, organismal fitness and increased risk of age-related diseases and death. Several hundred theories have attempted to explain this phenomenon. One of the most popular is the 'oxidative stress theory', originally termed the 'free radical theory'. The endocrine system seems to have a role in the modulation of oxidative stress; however, much less is known about the role that oxidative stress might have in the ageing of the endocrine system and the induction of age-related endocrine diseases. This Review outlines the interactions between hormones and oxidative metabolism and the potential effects of oxidative stress on ageing of endocrine organs. Many different mechanisms that link oxidative stress and ageing are discussed, all of which converge on the induction or regulation of inflammation. All these mechanisms, including cell senescence, mitochondrial dysfunction and microRNA dysregulation, as well as inflammation itself, could be targets of future studies aimed at clarifying the effects of oxidative stress on ageing of endocrine glands.

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Introduction

Ageing is a complex phenomenon that is still not completely understood. The process is characterized by a progressive decline in cellular function and organismal fitness, and an increased risk of age-related diseases and death. One of the most widely accepted theories to explain why organisms age and die was the so-called oxidative stress (or free radical) theory.[1] Reactive oxygen species (ROS) are produced mainly in the mitochondria, where aerobic metabolism takes place. The incomplete reduction of oxygen leads to the generation of different radical species such as the superoxide radical (O_2^-). This superoxide radical can then give rise to ROS such as hydrogen peroxide (H_2O_2)—a major contributor to oxidative damage. As the production of ROS seems to be an inescapable side effect of vital processes, particularly aerobic metabolism, a theory that explained ageing as a consequence of the oxidative damage was immediately appealing. Indeed, according to this theory, the random, unrepaired damage to macromolecules caused by ROS should be the leading driving force of ageing. In this paper, we will review the current knowledge of the effects of oxidative stress on the ageing of the different endocrine organs and tissues. A plethora of data indicate that oxidative stress and inflammation are interconnected, and ageing is now accepted to be characterized by a chronic, subclinical inflammatory state. We will therefore discuss the possibility that inflammation is the link between oxidative stress and ageing of endocrine tissues.

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Oxidative stress and endocrine systems

Hypothalamus, pituitary and pineal glands

The hypothalamic–pituitary axis controls many parts of the endocrine system. Ageing has been described as being associated with a progressive functional loss of several functions and body systems, including the hypothalamic–pituitary axis.[2,3] This progressive decline in function gradually develops into endocrine deficiency, which is potentially involved in human senescence.[2] For example, the levels of growth hormone (GH), insulin-like growth factor 1 (IGF-1), TSH and thyroid hormones progressively decrease with age in adults.[2,3]

According to a revised 'nitric oxide theory' of ageing,[4] an excessive production of free radicals and ROS in the central nervous system and its related glands, including the hypothalamic–pituitary axis, might be one of the most important factors in the ageing of these structures and the ageing process in general. In favour of this hypothesis, Kondo *et al.*[5] described a clear age-dependent accumulation of 8-hydroxy-2-deoxyguanosine, a major oxidative product, in the human pituitary gland. Similarly, Rodrigues *et al.*[6] found that during ageing of Wistar rats, the levels of free radicals in the hippocampus increased, in parallel with a reduced antioxidant capacity in both the hippocampus and the hypothalamus. Therefore, an imbalance between the production of oxidants and protective antioxidant systems in favour of an excessive accumulation of ROS might cause cellular oxidative damage in the hypothalamus and pituitary gland, as shown by the increase in the number of apoptotic cells during ageing, particularly for cells that secrete GH and TSH.[7] Interestingly, the oxidative stress caused by this imbalance might induce progressive age-related dysfunction

of the hypothalamic–pituitary axis not only through direct cellular damage, but also by alterations in protein function and by suppressing protein synthesis.

Lipid peroxidation can produce highly reactive aldehydes, such as malondialdehyde and 4-hydroxynonenal, which are able to bind covalently to several proteins as aldehyde–protein adducts. This deleterious process induces protein dysfunction and cellular damage. In 2011, Arguelles *et al.*[8] reported a decrease in levels of elongation factor 2, an essential factor for protein synthesis, in the hypothalamus and pituitary gland of rats during ageing. Oxidative stress is involved in alterations of elongation factor 2, such as the formation of adducts with malondialdehyde and 4-hydroxynonenal. In fact, the loss of elongation factor 2 that is observed during ageing is accompanied by a concomitant increase in levels of lipid peroxides of the carbonyl groups in proteins, which is a marker of ROS-mediated protein oxidation. This process could contribute to the reduced production of peptide hormones from the hypothalamic–pituitary axis that is observed with ageing.

In animals and humans, ageing is also associated with dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis, which leads to increased release of glucocorticoids from the adrenal cortex (Figure 1).[9] Although activation of the HPA axis is essential for adapting to a homeostatic challenge, chronic exposure to high levels of glucocorticoids can be harmful to human health and predispose the person to neurological, psychiatric, cardiovascular, metabolic and immune disorders.[10] The dysfunction of the HPA axis that is observed during ageing seems to be caused by a decline in the inhibition of the negative feedback of glucocorticoid levels over activity of the HPA axis. This feedback mechanism, which is regulated by glucocorticoid and mineralocorticoid receptors in the hippocampus, hypothalamus and pituitary gland, constitutes the most important control mechanism that modulates the HPA axis and its response to stress (Figure 1).[11,12]

The age-dependent increase in levels of glucocorticoids has been reported to be associated with chronic stress and cognitive impairment.[9,13,14] In fact, high concentrations of glucocorticoids over the long-term can be deleterious, particularly for the parts of the limbic system that are implicated in the regulation of cognitive and emotional processes. In Alzheimer disease, damage to neurons in the CA1 region of the hippocampus can induce dysregulation of the HPA axis that leads to increased cortisol release and cognitive decline.[13] These findings support the 'glucocorticoid cascade hypothesis'[14] and the 'glucocorticoid vulnerability hypothesis',[15] which suggest that impairment of the negative feedback control of the HPA axis in the aged rat (24 months old) leads to long-term exposure of the brain to glucocorticoids. The hypotheses state that exposure to subsequent insults then leads to damage to the hippocampus and prefrontal cortex and/or an enhanced neuronal vulnerability. These events result in an age-related cognitive impairment and a functional decline of the inhibitory control of the HPA axis.[14,15]

Key points

- Reactive oxygen species (ROS) are an inescapable by-product of oxidative metabolism and are believed to be involved in ageing, but they are also essential for several physiological functions
- Data indicate that the endocrine system is involved in the modulation of oxidative stress through the production of several hormones
- Oxidative stress also seems to have a role in the ageing of the endocrine system and in the pathogenesis of several endocrine diseases
- How oxidative stress causes ageing in endocrine tissues is unclear; in some tissues, inflammation is probably the link between the two processes
- ROS can induce inflammation directly by acting on transcription factors such as nuclear factor κB and indirectly by modulating other processes such as cellular senescence, mitochondrial dysfunction and microRNA production

However, the origin of the age-dependent dysfunction of the control of levels of glucocorticoids has not yet been fully defined. The levels of oxidative stress and inflammatory cytokines gradually increase with ageing, whereas the activity of antioxidant defences decreases. This phenomenon might be responsible for the overactivation of the HPA axis through hippocampal oxidative damage that leads to a decrease in the number of neurons expressing glucocorticoid receptor in the CA1 region during ageing (Figure 1).[16] In addition, long-term high levels of secretion of glucocorticoids has an important stimulatory effect on oxidative stress, mainly in the brain and heart, and increases the vulnerability of the hippocampus to other insults.[17,18] This finding implies that during ageing, the overactivation of the HPA axis, which is probably caused by oxidative stress, induces further oxidative stress and continuously stimulates the HPA axis through serious damage to the hippocampal pyramidal cells.[18] Furthermore, the continuous stimulation of the HPA axis can also induce inflammation, as peripheral mononuclear cells stimulated with corticotropin-releasing hormone increase their production of IL-6.[19] By contrast, centenarians have an increased level of glucocorticoids,[20] which could be considered an adaptive phenomenon in response to the stress present in physiological ageing.[21]

Several pituitary hormones and peripheral effectors (other than adrenocorticotropic hormone and glucocorticoids) can influence oxidative stress and longevity. TSH can result in oxidative stress through stimulating the production of thyroid hormones.[22] Accumulating evidence from *in vitro* and *in vivo* models indicates that activation of the GH/IGF-I system increases the production of ROS and decreases levels of antioxidants.[23,24] However, researchers have also demonstrated that IGF-I prevents apoptotic cell death induced by oxidative stress and has both anti-inflammatory and atheroprotective effects.[25,26] Although in *Caenorhabditis elegans*, *Drosophila melanogaster* and rodents, downregulation of GH/IGF-I signalling has been reported to extend survival considerably, data in humans are controversial.[27–31] Indeed, isolated GH deficiency and overproduction of GH in patients with acromegaly have both been associated with a reduced lifespan.[32] Interestingly, reduced levels of IGF-I bioactivity have been found in centenarians and the offspring of centenarians compared with the offspring of the matched-controls.[3]

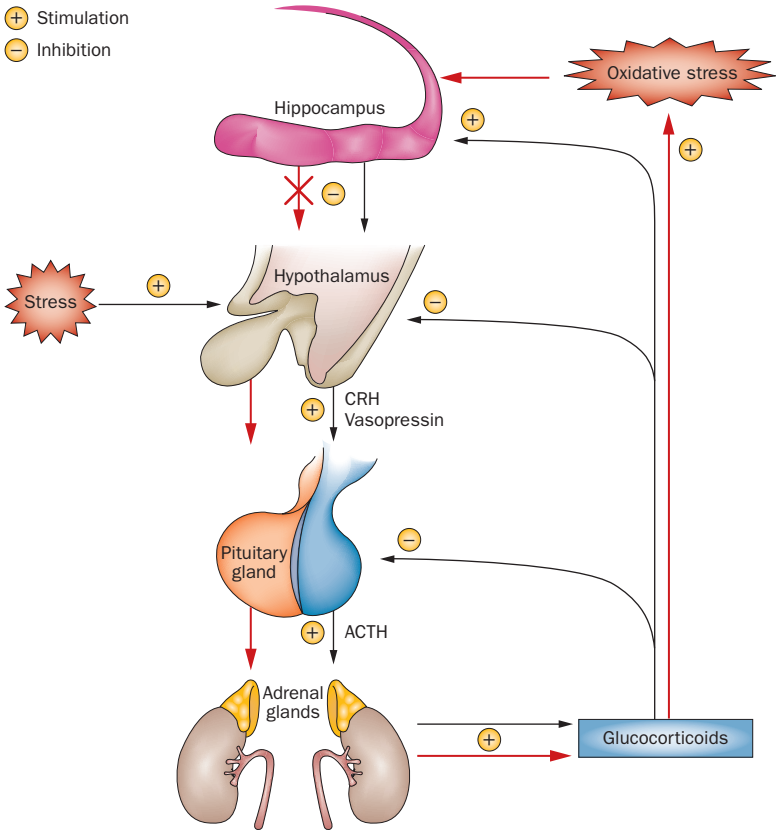

Figure 1 | Schematic diagram showing the potential role of oxidative stress in the progressive dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis observed with ageing. Under normal conditions (shown as black lines), the presence of a stressor stimulates the paraventricular nucleus of the hypothalamus to release CRH and vasopressin, which induce the release of ACTH from the anterior pituitary gland. ACTH stimulates the synthesis and release of glucocorticoids from the adrenal cortex. As a result of the deleterious effects of long-term exposure to glucocorticoids, a strict glucocorticoid-feedback mechanism, acting at the pituitary, hypothalamic and hippocampal levels, is fundamental to modulate the activity of the HPA axis. In particular, the activation of hippocampal glucocorticoid-receptor-expressing neurons exerts a potent inhibition of the HPA axis. During ageing (shown as red lines), dysregulation of the control of the activity of the HPA axis, probably secondary to an increase in hippocampal oxidative stress, stimulates glucocorticoid release, which in turn produces neural damage in the hippocampus, thus worsening the inhibitory control of the HPA axis. Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone.

The pineal gland is also able to modulate oxidative stress through the production of melatonin, a hormone with antioxidant and antiageing activities. Melatonin is a potent scavenger of ROS that has stimulatory effects on the antioxidant system. Finally, melatonin stabilizes cell membranes, thereby increasing their resistance to oxidative stress.[33–35] On the basis of these arguments, similarly to what has been reported for the pituitary gland and hypothalamus, excess production of ROS in the central nervous system might contribute to the age-related decline in function of the pineal gland, gradual decrease in nocturnal production of melatonin and to calcification of the gland.[4] Not surprisingly, the age-related decline in melatonin production and altered melatonin rhythms can contribute to the increased levels of oxidative stress observed in the elderly.[34,35]

Thyroid

The thyroid gland is able to produce large amounts of H_2O_2 for use in the synthesis of thyroid hormones (Figure 2). In the thyroid gland, H_2O_2 is produced by dual oxidases (DUOXs) sited at the apical membrane of the thyrocyte. Iodide is rapidly oxidized by thyroid peroxidase in the presence of H_2O_2 and incorporated into the tyrosine residues of thyroglobulin on the luminal side of the apical membrane. This step produces monoiodotyrosine and diiodotyrosine. Next, the coupling of two diiodotyrosine molecules generates T_4, while the combination of diiodotyrosine with monoiodotyrosine produces T_3, both processes are catalyzed by thyroid peroxidase. Thyroglobulin is internalized at the apical pole of thyrocytes, conveyed to endosomes and lysosomes and digested by proteases. After thyroglobulin digestion, thyroid hormones are released into the circulation.[36,37] Therefore, thyroid epithelial cells are constantly exposed to ROS, which are potentially toxic for cells. Furthermore, efficient protection of thyrocytes against excessive production of ROS through a sophisticated antioxidant system is crucial.[38] If the thyroid system is not properly regulated, an imbalance between ROS and antioxidants in favour of the oxidants, a phenomenon frequently observed with ageing, might induce several types of morphological and functional damage in the thyroid gland.

Ageing is associated with a decrease in the volume of the thyroid gland and in the levels of thyroid hormones secreted, as well as an increase in the prevalence of several thyroid diseases.[39–41] The latest studies seem to indicate that age-related subtle thyroid hypofunction (either as a result of a familial component or because of a reset of the thyroid function occurring between the sixth and the eighth decade of life) is related to longevity.[42,43] Although no clear evidence indicates that oxidative stress is responsible for the age-related morphofunctional changes of the thyroid gland, oxidative stress seems to be involved in the pathogenesis of thyroid autoimmune diseases (for example, Graves disease and Hashimoto thyroiditis) and thyroid cancer.[44]

The findings of several studies suggest that oxidative stress is involved in the pathophysiology of thyroid autoimmune diseases through a direct effect on the immune system.[45,46] Oxidative stress can alter the structure and antigenicity of self proteins through post-translational modifications, which trigger the development of autoimmune diseases (Figure 2).[47] Duthoit *et al.*[48] reported that exposure to fairly high concentrations of H_2O_2 induced thyroglobulin fragmentation in cultures of human thyroid cells. They hypothesized that autoantigen fragmentation through oxidative stress would be the initial event leading to a thyroglobulin autoimmune response (which can lead to thyroid autoimmune disease). Thyroid peroxidase might also cause similar events.[48] Another mechanism for increased autoimmunity is the raised expression of intercellular adhesion molecule 1 (ICAM-1) on thyrocytes in the presence of high levels of ROS (Figure 2).[45] ICAM-1 has a key role at an early stage in the onset of inflammatory responses.

Patients with Graves disease and Hashimoto thyroiditis have increased expression of ICAM-1 in their thyroid glands.[49] In support of the hypothesis that increased levels of ROS are involved in the pathogenesis of autoimmune diseases, evidence suggests that patients deficient in selenium who have autoimmune thyroid diseases benefit from selenium supplementation.[50,51] Selenium is part of the catalytic group within several selenoenzymes that have antioxidant activity.[50] Interestingly, the thyroid gland has the highest concentration of selenium of all tissues to protect thyroid cells from the oxidative stress induced by the synthesis of thyroid hormones.[50] As selenium levels decrease with ageing,[52] this process might leave the thyroid more vulnerable to oxidative stress.

Several groups of researchers have found an association between oxidative stress and thyroid cancer, reporting an increase in levels of oxidants and/or a decrease in antioxidant activity in patients with the disease.[53–58] The accumulation of excess ROS in the thyroid gland can cause DNA damage, resulting in mutagenic genetic alterations and promoting tumour initiation and development (Figure 2).[59] Through similar mechanisms, oxidative stress (observed with age in the thyroid gland and stimulated by deficiency in iodine or selenium, smoking or an increase in levels of TSH) might be involved in the pathogenesis of nodular goiter. If the antioxidant defence is not effective, this oxidative stress will cause DNA damage in the thyroid that is followed by an increase in the rate of spontaneous mutations and stimulation of proliferation of thyroid epithelial cells (Figure 2).[60]

The relationship between the thyroid gland and oxidative stress is further strengthened by data indicating a clear effect of thyroid hormones on oxidative metabolism. Thyroid hormones increase oxygen consumption via thermogenic activity and by stimulating mitochondrial respiration. This activity results in upregulation of the production of ROS, inducing oxidative damage of the membrane lipids in target tissues.[22,57] Thyroid hormones also seem to regulate the antioxidant defence system through the modulation of the synthesis and degradation of antioxidant enzymes and nonenzymatic antioxidants.[61–64]

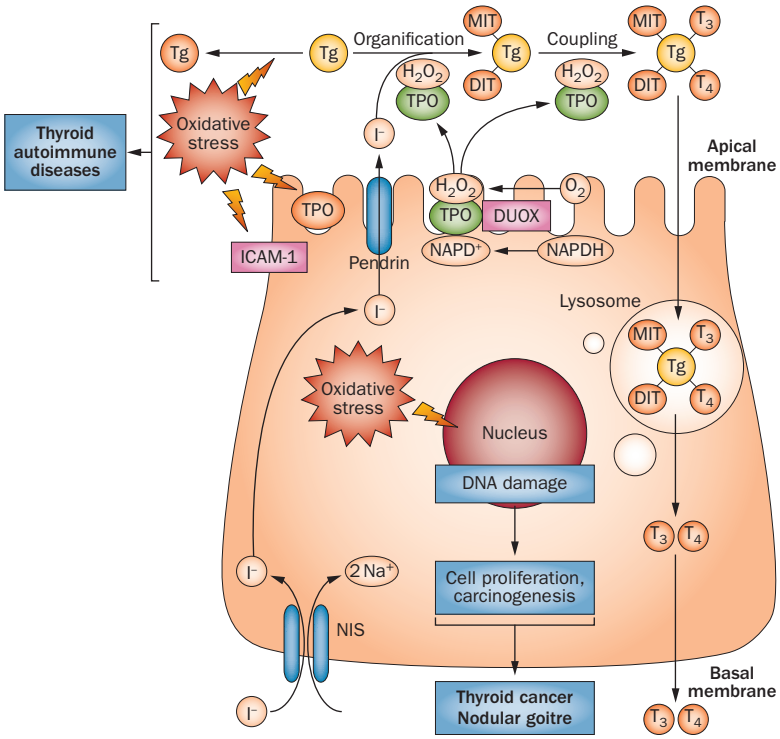

Figure 2 | Schematic representation of thyroid hormone synthesis in thyrocytes. I^- is actively transported into the thyrocyte through a NIS. This element enters into the follicular lumen from the cytoplasm via the transporter pendrin. In the colloid, I^- is oxidized by the TPO H_2O_2 system and is then used to iodinate tyrosyl residues in Tg, forming MIT and DIT. H_2O_2 is generated by the NADPH-dependent oxidase/peroxidase (DUOX). T_4 and T_3 are produced by coupling of iodinated tyrosyl intermediates (MIT and DIT), which are then endocytosed, hydrolysed in lysosomes and secreted into the bloodstream. Thyrocytes might accumulate oxidative damage as a result of constant exposure to H_2O_2 and/or an impairment of the antioxidant system during ageing. This phenomenon might induce morphological and functional damage, such as alterations in structure and antigenicity of Tg and TPO (ROS-modified molecules are shown in orange), responsible for several thyroid diseases, including those of an autoimmune or neoplastic nature. Abbreviations: DIT, diiodotyrosine; H_2O_2, hydrogen peroxide; I^-, iodine; MIT, monoiodotyrosine; NIS, sodium-iodide symporter; Tg, thyroglobulin; TPO, thyroid peroxidase.

Endocrine pancreas

The endocrine function of the pancreas deteriorates with age, which contributes to impaired glucose homeostasis. Ageing in humans is associated with both increased insulin resistance and decreased β-cell function,[65] which explains the strong association between the incidence of type 2 diabetes mellitus and advanced age.[66] All these mechanisms have oxidative stress as a common factor, and the relationship between oxidative stress, ageing of β-cells and diabetes mellitus has been widely studied in the past three decades.[67]

Oxidative stress has a major role in the onset and progression of type 1 and type 2 diabetes mellitus. Indeed, a number of risk factors for diabetes mellitus, such as increased age, obesity and unhealthy eating habits, favour a status of oxidative stress contributing extensively to β-cell dysfunction and/or death and impairment of insulin sensitivity.[68] ROS effect β-cell function differently according to levels of ROS and duration of exposure.[69] Short-term exposure to low concentrations of H_2O_2 derived from glucose metabolism is an important metabolic signal to elicit glucose-stimulated secretion of insulin.[70,71] 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.[72,73] In fact, β-cells are highly sensitive to oxidative stress because of their low antioxidant defence capacity.[74] During this detrimental process, mitochondrial dysfunction is a central contributor to the failure of β-cell function.[72,75]

Oxidative-stress-induced uncoupling proteins (UCPs) are anion mitochondrial carrier proteins that are expressed in the inner membrane of mitochondria. These proteins uncouple oxygen consumption by the

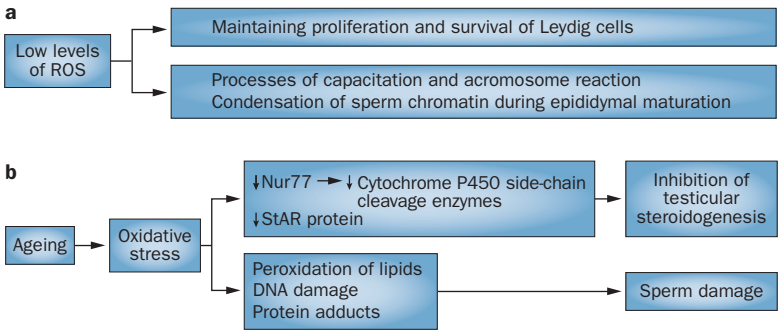

Figure 3 | Potential role of ROS on testicular physiology and testicular ageing. **a** | Low concentrations of ROS have several physiologically important roles such as modulation of Ras-ERK1/2 cascade, a crucial pathway for the proliferation and survival of Leydig cells; driving of capacitation and acromosome reaction processes; condensation of sperm chromatin during epididymal maturation and providing protection against oxidative DNA damage. **b** | A progressive increase in levels of ROS and/or a decline in the efficiency of antioxidant systems, observed with ageing, induce a state of oxidative stress in testes. Excessive production of ROS inhibits testicular steroidogenesis in Leydig cells by reducing the activity of cytochrome P450 side chain cleavage enzyme and expression of the StAR protein. An excess in seminal ROS levels has toxic effects on both sperm quality and function through the peroxidation of lipids, the induction of oxidative DNA damage and the formation of protein adducts. Abbreviations: ROS, reactive oxygen species; StAR protein, steroidogenic acute regulatory protein.

respiratory chain from ATP synthesis and serve as a defence mechanism against the deleterious effects of high levels of ROS. In β-cells, oxidative stress induces activation of UCP2. This activation results in protons leaking across the inner membrane of mitochondria and decreases production of ROS by controlled negative feedback. However, activation of UCP2 also reduces ATP synthesis and content in β-cells, which impairs insulin secretion. In addition, chronic oxidative stress causes cardiolipin peroxidation in mitochondrial membranes, which in turn causes cytochrome c to be released into the cytosol and caspase 3 to be activated, thereby inducing apoptosis in β-cells.[72,75] Production of ROS can also lead to injury of β-cells through destruction of lipids in the cell membrane and cleavage of DNA.[76] Interestingly, these deleterious processes are exacerbated with ageing not only by accelerated production of ROS, but also by a decreased proliferative activity and enhanced sensitivity to glucose-induced apoptosis of β-cells.[77]

Oxidative stress also contributes to the increase in insulin resistance observed with ageing, which affects the insulin signalling cascade through several mechanisms. Insulin resistance is increased by inducing serine/threonine phosphorylation of insulin receptor substrate, disturbing cellular redistribution of insulin signalling components, decreasing transcription of *GLUT4* and impairing mitochondrial activity.[68]

Gonads

Endogenous ROS have important roles in the modulation of several physiological reproductive processes in women and men. These ROS mainly originate from inflammatory cells (such as macrophages and neutrophils), which are recruited to the ovary in response to the luteinizing hormone surge (which triggers ovulation).[78,79] ROS are also produced by steroidogenic cells through the activation of the mitochondrial P450 system.[80] In ovarian tissue, ROS are necessary for oocyte maturation, ovarian steroidogenesis and the function and disruption of the corpus luteum.[78,79] The growth and maturation of oocytes is controlled by the oxidative system. The selection of the dominant oocyte, including during meiosis I, is stimulated by an increase in levels of ROS and is inhibited by antioxidants, whereas the progression of meiosis II is promoted by antioxidants.[78,79] ROS production by the preovulatory follicle is considered an indispensable preovulatory signalling event and an inducer of ovulation.[81] ROS are also involved in the mechanisms of luteolysis through the induction of apoptotic cell death and affect the production of progesterone by impairing luteinizing hormone receptors or by inhibiting the translocation of cholesterol to the mitochondria or cytochrome P450scc enzyme activities.[82] In addition, antioxidants seem to have an important role in preventing regression of the corpus luteum rescue if pregnancy occurs.[79,82]

Ovarian ageing is characterized by a gradual decrease in both the quantity and the quality of the pool of oocytes and follicles, with the menopause as the final step of this process.[83] The most relevant theory for ovarian ageing, first proposed by Tarin,[84] implies that oxidative stress is associated with repeated ovulation. The cyclical production of ROS over many years might lead to cumulative DNA damage that contributes to ovarian ageing, an increasing risk of ovarian diseases and complications during pregnancy.[78,79] Indeed, old age was associated with increased levels of ROS and/or a weakening of antioxidant defences in oocytes, granulosa cells and follicular fluid.[85–87] Tatone *et al.*[88,89] have suggested a role for long-term exposure to advanced glycation end products (AGEs), gradually accumulated over many years, in the ovarian ageing process. The accumulation of AGEs during a woman's reproductive lifespan might cause mild oxidative damage in primordial follicles and ovarian stroma vessels.[88] In fact, AGEs are potent stimulators of oxidative stress through interaction with specific cellular receptors and by impairing vascular function.[90,91] Evidence that the disruption of the balance between pro-oxidants and antioxidants has an important role in the pathogenesis of polycystic ovary syndrome[92,93] and ovarian cancer[94–97] provides additional support for the interaction between oxidative stress and ovarian ageing.

Endogenous ROS also have a physiological role in testes (Figure 3a). ROS serve as a positive signal for maintaining a functional population of adult Leydig cells.[98] Low levels of ROS are needed to regulate the maturation and function of spermatozoa, including sperm capacitation and acrosome reaction.[99,100] Despite the low oxygen tensions that characterize the testicular environment, the testes are highly vulnerable to oxidative stress, mainly as a result of the abundance of unsaturated fatty acids, the local production of ROS in the mitochondria and of several enzymes, including xanthine oxidase, NADPH

oxidase and cytochrome P450.[101] To support a correct Leydig cell steroidogenesis and spermatogenesis, a complex battery of enzymatic and non-enzymatic antioxidant defence systems works to prevent damage from lipid peroxidation.[101]

Testicular ageing is characterized by reduced serum levels of testosterone and by a decline in spermatogenesis.[102,103] An increase in levels of ROS and/or a decrease in levels of antioxidants, which are observed with ageing in testicular tissue, have been proposed as possible explanations for the age-related decline in the synthesis of testosterone and spermatogenesis (Figure 3b).[104–106] Reduced serum levels of testosterone seem to depend on a progressive inhibition of steroidogenesis observed in Leydig cells with ageing (Figure 3b).[103,104] ROS inhibit testicular steroidogenesis in Leydig cells by suppressing cholesterol transfer into the mitochondria through a reduction in levels of the StAR protein, and by decreasing the expression of genes that encode steroidogenic enzymes via the suppression of Nur77 transactivation.[107] In addition, Leydig cells, as a result of their location in the testicular interstitium, are particularly vulnerable to extracellular sources of ROS because of their contiguity to testicular interstitial macrophages, which represent about 25% of the interstitial cell population in the testes of mammals.[108,109]

The decline in male fertility with ageing is associated with increasing oxidative stress and the accumulation of oxidative damage to the seminiferous tubules through the peroxidation of lipids, the induction of oxidative DNA damage and the formation of protein adducts (Figure 3b).[110] Spermatozoa are particularly vulnerable to oxidative damage because their cell membranes contain high levels of polyunsaturated fatty acids, which can be oxidized as a result of high levels of ROS. This detrimental process that is observed with ageing can induce impairment of sperm motility and decrease sperm viability.[99,111]

Sex hormones might also influence oxidative stress. Mitochondrial oxidative stress is higher in men than in women.[112] Indeed, the higher levels of oestrogens in women than in men protect women against ageing by upregulating the expression of genes related to antioxidants and longevity.[112,113] This protective role of oestrogens has been confirmed by the finding that oxidative stress in ovariectomized rats increased to the same levels as are seen in males.[113] In addition, oestrogen replacement therapy in ovariectomized rats restores peroxide levels to those found in normal females.[113] Conversely, androgen deprivation and androgen receptor activation both seem to induce oxidative stress.[114] These observations contribute to the explanation of the longer lifespan of females than males in many vertebrate species.[112]

Adrenal glands

Ageing of the adrenal glands is characterized by dysregulation of the HPA axis and a progressive decline in the adrenal production of androgens.[115,116] Excessive generation of ROS and oxidative damage might be responsible for the age-dependent loss of androgenic function, especially by damaging cellular membranes involved in mitochondrial transport of cholesterol and steroidogenesis through lipid peroxidation. Indeed, the risk of damage from lipid peroxidation is especially high for steroidogenic cells because these cells, which are rich in lipids, use molecular oxygen and produce high levels of ROS through cytochrome P450 systems.[117] Interestingly, oxidative stress-mediated inhibition of adrenal steroidogenesis during ageing is accompanied by a selective activation of p38 MAPK, a negative determinant of adrenal steroidogenesis.[118] In addition, evidence indicates that ageing modifies the expression of two oxidant-sensitive transcription factors—AP-1 and nuclear factor κB (NF-κB).[119,120]

Adipose tissue

White adipose tissue (WAT) is an important source of cytokines, chemokines and adipokines, through which WAT modulates a series of biologic activities, including appetite, energy expenditure, insulin sensitivity, the endocrine and reproductive systems and bone metabolism.[121] In particular, it has been observed that inflammation and immunity are affected by factors produced by WAT. As an example, as much as 30% of circulating IL-6 is estimated to be produced by WAT, particularly visceral WAT.[122,123] Tumour necrosis factor is also released by WAT, and its production is increased in people with obesity.[124,125] These cytokines (IL-6 and tumour necrosis factor) are produced by infiltrating macrophages, which are a normal component of WAT, but also by adipocytes. Other proinflammatory components such as CCL2 (also known as monocyte chemoattractant protein 1), CCL3 (also known as macrophage inflammatory protein 1α) and IL-8 are also produced by WAT.[126–128] Among the adipokines, leptin, resistin, RBP4, lipocalin 2, complement factor D (also known as adipsin), NAMPT (also known as visfatin) and ANGPTL2, as well as IL-6, IL-18, CCL2 and CXCL5, are known to exert proinflammatory effects, whereas adiponectin and SFRP5 are considered anti-inflammatory,[121,129] even though the data are not concordant.[130] Production of these cytokines affects diseases with an inflammatory pathogenesis, among which type 2 diabetes mellitus is of major interest because of its links with obesity. Indeed, proinflammatory molecules such as tumour necrosis factor are known to have direct effects on insulin sensitivity, as they are able to induce phosphorylation of the insulin receptor, thereby inhibiting insulin signalling.[131]

In a study where centenarians' offspring were characterized by better health than age-matched control individuals whose parents were not long-lived, no differences were found in terms of plasma levels of adipokines.[132] However, when focusing the analysis on patients with the metabolic syndrome, centenarians' offspring seemed to be healthier and more functionally fit and had lower levels of resistin than offspring of not-long-lived people. This finding suggests an important role for adipokines in determining the health status of old (aged 65–77 years) people. Not surprisingly, the

production of these inflammatory mediators has been linked to oxidative stress. Indeed, it has been reported that adipocytes can generate ROS, and that the increased production of such ROS, especially during hypoxia, is linked to dysregulated expression of adipokines, including adiponectin, plasminogen activator inhibitor 1, IL-6 and CCL2.[133] Accordingly, researchers have observed that physical exercise decreases the expression of inflammatory adipokines through a reduction in the level of oxidative stress.[134] Interestingly, CCL2 is also involved in adipogenesis, via ZC3H12A (also known as MCPIP) that acts by inducing the production of ROS and reactive nitrogen species.[135] Therefore, WAT produces inflammatory mediators via a mechanism that includes generation of ROS, and some of these inflammatory molecules then induce adipogenesis through production of ROS. In fact, it has been recognised that not only adipocyte hypertrophy but also hyperplasia contributes to increased levels of WAT in adults.[136] Hence, ROS seems to have a central role in WAT-generated inflammation and adipogenesis, but this role has been questioned by the observation that the ablation of *Nrf-2*, which is involved in the antioxidant response, in mice impairs adipogenesis and protects against diet-induced obesity.[137]

The production of ROS and consequently of inflammatory compounds by both infiltrating macrophages and adipocytes is considered an early stage of metabolic diseases such as type 2 diabetes mellitus. Interestingly, a plethora of microRNAs has been associated with the inflammatory phenotype found in dysfunctional adipocytes and with the increased adipogenesis present in obesity.[138,139] Some of these microRNAs are involved in the regulation of inflammation. For example, miR-21 inhibits the TGF-β signalling pathway; TGF-β is not only an anti-inflammatory cytokine, but is also involved in the inhibition of adipogenesis.[140] Interestingly, the circulating level of miR-21 has been found to be increased in old people (aged ~80 years old), but decreased in centenarians.[141] A number of microRNAs exert similar effects in both WAT and vascular tissue.[138] Assuming that miR-21 can have the same effects on WAT and vascular tissue, it could be hypothesized that a decreased level of miR-21 is one of the mechanisms through which inflammation at the level of endothelia is controlled in centenarians, accounting for the low rate of cardiovascular diseases in these exceptional individuals.

In addition, WAT is now known to be able to produce inflammatory mediators as a metabolic reaction to excess intake of nutrients, and this type of inflammation has been termed 'metaflammation'.[142] In mice, excess fat in the diet has been linked to suppression in WAT of *de novo* synthesis of fatty acids,[143] among which palmitoleate seems to be of particular importance.[143] Palmitoleate exerts anti-inflammatory effects in adipocytes and its circulating form promotes insulin sensitivity in muscle and liver.[143] Weight control in patients undergoing a dietary intervention has been correlated with high levels of palmitoleate and other unsaturated fatty acids in WAT,[144] which suggests that signalling processes that are mediated by fatty acids might also exist in humans. At present, whether ROS can influence the production of these mediators is unclear.

WAT is the only endocrine tissue that does not undergo age-related involution but rather increases with age. Fat mass, BMI and percentage of body fat are known to increase from age 20 years and level off at ~80 years.[145] Other than the effect on metabolic diseases such as type 2 diabetes mellitus, the increase in the amount of WAT is known to be associated with decreased muscle force and increased disability, morbidity and mortality.[146] Therefore, fat and muscle tissues seem to have an intense crosstalk that impedes muscle function. Skeletal muscle produces its own cytokines—myokines—and can thus be considered as a *sui generis* endocrine tissue. The prototypic myokine is IL-6, which is important for proper muscle metabolism.[147] Stress signals leading to the production of IL-6 in muscle include oxidative or nitrosative stress, together with damaged or unfolded proteins, hyperthermia or energy imbalance. For these reasons, skeletal muscle has been proposed to be a sensor and responder to stresses, among which oxidative stress can have a prominent role as a result of the high level of oxygen consumption that takes place in this tissue.[148] Other myokines such as MSTN (myostatin), LIF, IL-7, IL-15, BDNF, IGF-1, FGF-2, FSTL-1, FNDC5 (irisin) and SPARC have many different autocrine, paracrine and endocrine effects on target organs, including WAT.[149] Although muscle ageing is widely studied in terms of loss of force, much less is known about its possible age-related modifications as an endocrine tissue, and about the interconnections between oxidative stress and myokines. This topic will probably represent one of the new frontiers in endocrinology research.

Oxidative stress is believed to be involved in the development of obesity and metabolic syndrome and it can contribute to the neuron damage observed in several neurodegenerative disorders for which metabolic syndrome is a primary risk factor.[150,151] In this respect, lipid peroxidation has a crucial role. The resulting breakdown products from lipid peroxidation, mostly aldehydes (malondialdehyde, 4-hydroxynonenal, hexanal and acrolein), adversely affect proteins involved in the regulation of cellular ion balance, energy homeostasis, cell proliferation, cytoskeletal structure and neurotransmission. These effects can compromise cellular function and lead to cell death.[150,152]

ROS, inflammation and ageing

Despite its great popularity, the free radical theory of ageing has been questioned by experimental evidence that has accumulated in the past few years.[153] For example, overexpression of enzymatic antioxidants such as superoxide dismutase seems to increase the lifespan of the worm *C. elegans*, but does not protect the worm against production of ROS.[154] Mice with alterations in genes involved in antioxidant systems do not show notable modifications of lifespan when kept in optimal husbandry conditions. These findings suggest that oxidative stress does not affect longevity. However, when these transgenic and/or knockout mice were tested

using models that develop various types of age-related pathology, they show alterations in progression and/or severity of pathology, which suggests that oxidative stress has a role in determining how many months or years an animal is disease-free for, rather than effecting lifespan.[155] Nevertheless, less pervasive, non-genetic manipulations known to improve longevity, such as calorie restriction and physical exercise, actually result in the production of ROS in experimental models,[156] a finding that is at variance with what is predicted by the ROS theory of ageing.

In *Homo sapiens*, the production of ROS is tightly controlled by a plethora of antioxidant systems.[157] Arguably, the allocation of resources for antioxidant defences has probably already evolved to a maximal level in long-living primates and humans, thus complicating any intervention against oxidative stress and probably minimizing the effect of any further supply of antioxidants. Available data on the variations with age of antioxidant defence mechanisms in humans do not arrive at a definitive conclusion, as some authors reported an increase or decrease in the activity of the mechanisms with ageing.[158,159]

Even more importantly, ROS qualify as fundamental second messengers that are important not only for a series of general biologic processes, such as the cell cycle,[160] metabolism, differentiation and cell survival,[161] but also for the above-mentioned specific differentiation and signalling processes in several endocrine organs. Therefore, the complete elimination or unspecific consistent downregulation of ROS is definitely not advantageous for the organism. Accordingly, on many occasions, clinical trials with antioxidants such as β-carotene, vitamin A and vitamin E have actually reported an increase in mortality.[162] Is oxidative stress therefore to be ruled out as a possible cause of ageing, as proposed?[153] We do not think that the oxidative theory of ageing is dead. Instead, we here propose that the oxidative theory of ageing is to be complemented and integrated with the inflammatory theory of ageing. Indeed, inflammation has all the characteristics to be a possible link capable of integrating oxidative stress and ageing.

A major characteristic of ageing seems to be a mild but constant increase in the production of a variety of proinflammatory mediators, which leads to a complex status of chronic, sterile, subclinical inflammation that we propose to term 'inflamm-ageing' (Box 1).[163–165] The majority of the age-associated diseases, such as type 2 diabetes mellitus, neurodegeneration, cancer, osteoarthritis, autoimmune and cardiovascular diseases, have an inflammatory background.[166] Thus, it can be surmised that unnecessary inflammatory responses characterize and probably cause the ageing phenotype. Inflammation is intrinsically linked to oxidative stress, as ROS can directly or indirectly activate transcription factors such as NF-κB and AP-1 that can promote inflammation.[167] A schematic picture of the molecular connections between oxidative stress and inflammation is provided in Figure 4. Of note, transcription factors such as NF-κB are also important for antioxidant and pro-survival cellular responses. Nevertheless, as mentioned above, whether these antioxidant responses decrease or increase with ageing is still debated, whereas the production of ROS is known to increase, thus tilting the redox balance toward a pro-oxidant state.

Cellular senescence, a stress response that suppresses tumours and is also associated with ageing, entails the acquisition of a phenotype characterized by the secretion of proinflammatory proteins, termed SASP (senescence-associated secretory phenotype), and might be an important additional contributor to chronic inflammation.[168] In some tissues, cell senescence can be induced by oxidative stress.[169] Therefore, ROS might possibly induce organ ageing via the induction of cell senescence, which might also be the case in many, if not all, endocrine tissues. Considering the present lack of knowledge regarding this specific topic, it is envisaged that studies in this direction will be one of the objectives of endocrinology research in the next few years.

Box 1 | Inflamm-ageing

Human ageing is characterized by an increased amount of circulating proinflammatory mediators, such as C-reactive protein and IL-6.[184] Our group proposed to indicate this phenomenon as 'inflamm-ageing'.[163–165] Should inflamm-ageing be a major (primary or secondary) cause of ageing, patients with low inflammatory responses would have a survival advantage. Centenarians can be considered the best example of successful ageing, but quite paradoxically they also have signs of inflammation and decreased antioxidant defences.[157,185–189] Despite this apparently unfavourable situation regarding inflammation and oxidative stress, these exceptional individuals avoided or consistently delayed diseases such as type 2 diabetes mellitus, cardiovascular diseases or invasive cancer.[190–192] These data in centenarians, as well as a plethora of other data in the elderly, lead to the important conclusion that inflamm-ageing and oxidative stress are universal phenomena associated with human ageing but paradoxically compatible *per se* with longevity, either disease-free or disease-associated. However, the cytokine-receptor system can be quite complex, and in some cases, lead to the opposite results (proinflammatory or anti-inflammatory responses), as in the case of IL-6 receptors, IL-6Rα and gp130.[193] Therefore, it is important to check which one of these receptors is expressed on endocrine tissues to understand which response is the effect of IL-6 on such tissues.

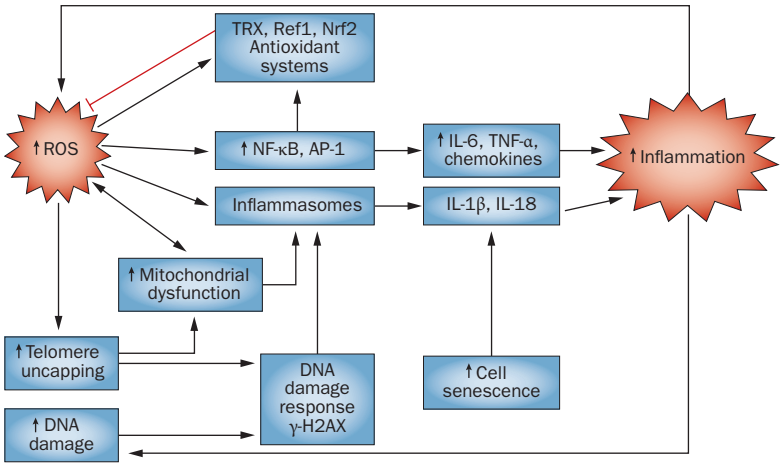

Figure 4 | Schematic representation of the connections between oxidative stress and inflammation and their modification during ageing. Small upright arrow: increase with age.[164,167,171,201–205] Abbreviation: ROS, reactive oxygen species.

Box 2 | The inflammasome

A molecular link between production of reactive oxygen species (ROS) and inflammation is represented by inflammasomes. In fact, pattern recognition receptors such as the nucleotide-binding domain leucine-rich repeat-containing receptor family (NLR), when triggered by a variety of sterile (molecules derived from the host or environment) or pathogen-associated activators, can stimulate the assembly of complexes called inflammasomes.[194] These inflammasomes promote the maturation of proinflammatory cytokines such as IL-1β and IL-18. The production of ROS can activate the NLRP3 inflammasome,[195] and the redox status of the cell regulates the processing and secretion of IL-1β.[196] ROS can induce DNA breaks that trigger the DNA damage response, which in turn activate AIM2 inflammasomes.[197,198] Thus, ROS can in some way activate the machinery that leads to the production of proinflammatory compounds and therefore to inflammation. Whether inflammasome activation is associated with inflamm-ageing is not yet clear, but it could be involved in many diseases that have an inflammatory pathogenesis, such as type 2 diabetes mellitus, autoimmune thyroid diseases, atherosclerosis, colorectal cancer and other inflammatory bowel diseases.[199,200]

Furthermore, ROS can damage DNA and thus elicit a DNA damage response, in particular via the ataxia telangiectasia mutated pathway, which seems to be preferentially activated by DNA double strand breaks and has been shown to serve as a sensor of oxidative stress.[170] The DNA damage response can actually trigger inflammation via the production of IL-6 (Figure 4).[171–175] These cytokines can induce DNA damage in bystander cells, which in turn will produce other cytokines, thus amplifying the original signals.[176]

ROS (and the DNA damage response) can also induce the maturation of the proinflammatory IL-1β, mediated by supramolecular complexes called inflammasomes (Box 2). Given all these connections between oxidative stress and inflammation, and given the fact that oxidative stress increases with age,[177–179] a continuous production of ROS for a period of time much longer than that determined by natural selection could possibly be among the leading causes of inflamm-ageing. However, major questions such as the link (or links) between ROS, inflammation and the activation of inflammasomes remain unanswered (Figure 4). Similarly, whether inflamm-ageing affects the endocrine system, and the magnitude of this effect, and whether inflammation could be the missing link between oxidative stress and ageing of endocrine tissues and organs is largely unclear. Specific studies to address this question are urgently needed.

As we argued regarding inflamm-ageing and pro-inflammatory and anti-inflammatory mediators,[164,165] we can hypothesize that what matters during ageing is the complex cell-specific, organ-specific and tissue-specific balance between the physiological and the pathological role of ROS and reactive nitrogen species. The most severe pathological effects of inflamm-ageing and oxidative stress can be largely independent of the total amount of proinflammatory mediators or oxidized products that can be measured at the systemic level, but the effects could be associated with the local balanced or unbalanced amounts of the mediators produced at the different, specific anatomical districts.[165,180] This hypothesis that is specific to cells, tissues or organs fits with the above-mentioned data on miRNAs, whose production is often specific to the tissue and disease.[181,182] Similar considerations can be applied to the cell-specific, organ-specific and tissue-specific balance between oxidants and antioxidants and to the interaction between inflammatory and oxidative responses. Accordingly, an integrated systems biology approach of inflammation and oxidative stress in ageing animals should be pursued to identify targets for drugs that are specific to cells, tissues or organs and are capable of exerting focused anti-inflammatory and antioxidative activity.

Conclusions

From an evolutionary perspective, it can be assumed that the physiological role of inflammation and the production of reactive oxygen and nitrogen species is not to cause ageing of the organism, as organisms cannot survive without them, but that these species are involved in ageing and age-associated diseases. Therefore, in the next few years, it will be necessary to investigate the following critical topics: which parts or components of inflammatory responses and oxidative stress must be switched off to delay ageing and avoid or postpone age-related pathologies; and whether the inflammatory reactions are causal for the ageing of the endocrine system itself and of the whole body, considering the crucial importance of hormones and immune responses in orchestrating the body's responses to all types of stressors.

Another challenge that is now appearing on the horizon of scientific research is the study of the effect of prenatal stresses on the ageing process and susceptibility to diseases over a person's lifespan. Context-inappropriate and time-inappropriate exposure to various stresses (including inflammatory and oxidative stress) during intrauterine development have been proposed to alter telomere biology.[183] The effects of prenatal stresses are probably not limited to telomeres and can have a more general spectrum of action, including in endocrine organs. This topic deserves specific investigations to connect early life events, oxidative stress and ageing of the endocrine system.

Review criteria

This Review was based on the authors' personal collection of publications and conference abstracts concerning oxidative stress and the ageing endocrine system, as well as mechanisms of human ageing and longevity. In addition, we performed a search for original articles and reviews published up to October 2012 using PubMed. The search terms used were "oxidative stress", "ROS", "ageing", "aging", "endocrine system", "hypothalamus", "pituitary", "thyroid", "endocrine pancreas", "beta cells", "ovary", "testis", "adrenal", "adipose tissue", "inflammation", "microRNA", "mitochondria", and "cell senescence". All papers identified were English-language, full-text papers. We also searched the reference lists of identified articles for further papers.

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Author contributions

G. Vitale and S. Salvioli researched data for the article. All authors contributed to discussion of the content, writing the article and reviewing and editing the manuscript before submission.