Sex differences in neuromuscular ageing: the role of sex hormones

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Abstract

Males and females experience different trajectories of neuromuscular function across the lifespan, with females demonstrating accelerated deconditioning in later life. We hypothesise that the menopause is a critical period in the female lifespan, during which, the dramatic reduction in sex hormone concentrations negatively impacts synaptic input to the motoneuron pool, as well as motor unit discharge properties.

Summary

The menopausal transition accelerates neuromuscular ageing in females compared to males.

Key Points

- Sex hormones influence central nervous system properties, with either neuro-excitatory or inhibitory actions.
- Premenopausal, eumenorrheic females experience cyclical changes in the neural excitation/inhibition balance across the menstrual cycle. This affects the firing rates of motor units that control muscle contraction.
- Females exhibit an exaggerated decline of neuromuscular function in later life, which may be a consequence of the drastic reduction in sex hormone concentrations following the menopausal transition.
- This review proposes the hypothesis that the rate of neuromuscular ageing is accelerated by the menopause in females, whereas males demonstrate a more gradual decline. This is potentially a contributing factor to the health-survival paradox whereby females live longer than males yet spend greater time in poor health.

Key Words:

Electromyography, female, frailty, male, motoneuron, gender, endocrine

Introduction

Neuromuscular ageing refers to the changes within the nervous system and skeletal muscle that contribute to impaired motor performance in later life. It is established that older adults experience reductions in maximal strength, power, and contractile speed, as well as greater fatigability than younger adults (1). The determinants of these age-related declines are multi-factorial and contribute to a heterogeneity in the ageing process between individuals (2). One aspect of neuromuscular ageing that is relatively under researched is the influence of biological sex, and the associated hormonal differences between males and females across the lifespan. Indeed, females typically live longer than males but spend a greater proportion of their life in poor health. For example, females have a higher frailty index score, with an increasing effect size into older age (3). Understanding and combating the health survival paradox between biological sexes is a public health priority, and it is imperative that future research seeks to address the disproportionate detrimental effects in older females. As such, this review aims to highlight potential mechanisms that underpin sex differences in neuromuscular ageing, with a focus on neuro-endocrine interactions. It should be noted that within this review sex is presented as male/female, however, the authors appreciate the evolving awareness of differences in sex development, in which the development of sex is not within the dichotomy of male/female (4).

Sex differences in hormonal changes across the lifespan

Oestrogen and progesterone are the primary female sex hormones and their release into the general circulation is mediated by the hypothalamic pituitary adrenal axis. Oestrogen refers to a group of steroid hormones of a similar structure which are primarily produced in the female ovaries and comprise estrones, estriol and estradiol-17β. Estradiol is the most prominent oestrogen within humans and is primarily responsible for reproductive function and the development of primary and secondary female sex characteristics, exerting its genomic effects (5). Oestrogens also regulate, directly and indirectly, a number of functions throughout both males and females such as energy metabolism, insulin sensitivity and substrate utilisation

acting through specific receptors (ERs) (6). Progesterone, also produced by the ovaries, is secondary to oestrogen and is the predominant form of the progestogen sex steroids. Progesterone can act antagonistically to oestrogen, blocking the ER receptor sites, and has other effects on the central nervous and vascular system (7). The ability of the oestrogen and progesterone to impact other physiological systems, other than development of sex characteristics, demonstrate the non-genomic capacity of these hormones.

Throughout the majority of adulthood, most females experience a cyclical pattern of sex hormone fluctuations through the menstrual cycle, a natural process occurring in the reproductive years between puberty and menopause. A 'normal' (eumenorrheic) menstrual cycle lasts 22–35 days and is primarily characterised by cyclical fluctuations of oestrogen and progesterone, although a highly heterogeneous process (8). Following menstruation, circulating oestrogen concentrations (~150 pmol/L) typically rise to a peak around day 10-14 (670 pm/L) followed by a slow decrease over the following 5 days (9). The latter ~ 14-day luteal phase is characterised by a gradual increase in progesterone, peaking around day 21 (progesterone levels ~ 20-55 nm/L and oestrogen ~500 pm/L) and returning to base levels at ~ day 28 (0.3-2 nm/L). Given the fluctuating nature of the hormones throughout a menstrual cycle, and the potential influences of oestrogen and progesterone on health and neuromuscular performance, it is not surprising that such a topic has come to the forefront of physiology research recently. Despite this, existing data on physiological changes in response to hormonal fluctuations is not substantial enough to effectively produce sex-specific recommendations for exercise, at any point across the lifespan (10).

Pregnancy drastically alters the course of naturally occurring hormones. From the time of conception, oestrogen and progesterone continue to rise, decreasing again following birth (11). Two further hormones are notable here; human chorionic gonadotrophin hormone (hCG) and human placental lactogen (hPL), both of which are produced within the placenta (11). However, the wider implications of this changing hormonal profile through pregnancy on other

physiological systems is relatively unknown and more research in this area is needed. Opposingly, the use of hormonal contraceptives, taken acutely or chronically, will also disrupt the adult hormonal cycle. These interventions provide exogenous forms of oestrogen and/or progesterone, creating an unsuitable hormonal environment for conception, reducing levels of endogenous hormones significantly.

Menopause marks the end of a female's reproductive life, prior to which they transition through perimenopause; characterised by irregular cycles of menstruation and ovulation, coinciding with high variation in levels of oestrogen and progesterone. The menopause is defined as the lack of a menstrual cycle for 12 consecutive months, resulting in a significant reduction in oestrogen and progesterone, which then remain at their respective nadirs until end of life (12). During the menopausal transition, the majority of females experience an array of vasomotor symptoms such as hot flashes, night sweats, brain fog and resulting sleep disturbances (13). Hormonal replacement therapy (HRT) has become a popular intervention to mitigate negative consequences of the menopause and involves administering exogenous synthetic hormones to counteract the hormonal losses, with ~1 million females currently using HRT in the UK. Although the use of such medication may mitigate some of the experiences of the transition through the menopause this will not continue beyond cessation of medication (14). Furthermore, the full implication of long-term use is not yet fully established. Prescriptions of low dose HRT demonstrate beneficial responses in relation to improvements of bone mineral density (15), and has shown to be effective in decreasing the number, severity and duration of hot flushes (16), and is associated with a better quality of life (17). However, no known studies directly assessing beneficial and detrimental effects of HRT on the mechanistic properties of the neuromuscular system.

Comparatively, the male hormonal milieu is more stable across the adult lifespan. Testosterone (T) is the primary male sex hormone and has numerous roles in regulating secondary sex characteristics. It is measured in its free and total form, both of which are known

to decrease with age (18). Testosterone has numerous effects in skeletal muscle with a widely noted facilitation of hypertrophy (19), with exogenous supplementation in older non-hypogonadal males being effective in promoting beneficial muscle adaptation (20). Dihydrotestosterone (DHT) is synthesised from testosterone and is the more powerful naturally occurring androgen, with around 3-6 times higher biopotency than testosterone (21). The precursors of testosterone; dehyrdroepiandrosterone (DHEA) and its sulphate derivative (DHEAS), have been shown to gradually decrease with ageing in men (22). Comparatively, the ratio of DHT: Free testosterone is initially raised in childhood, then declines after puberty and remains relatively unchanged from the ages of 20 years until end of life (21).

There are clear disparities in hormonal profiles between the sexes. The female hormonal environment changes at a number of time points throughout the lifespan, whereas the male environment is more predictable. Whilst hormonal environments are dominated by sex specific hormones (oestrogen and progesterone for females, testosterone for males) there are still levels of these hormones within the opposing sex, albeit present in much lower levels and relatively stable throughout the majority of adulthood, decreasing gradually in older age. These sex hormones are able to cross the blood-brain barrier due to their high lipid solubility, potentially influencing excitatory and inhibitory processes in the central nervous system (CNS) (23). Peripherally, they also act directly on specific receptors to initiate genomic and non-genomic effects on skeletal muscle (24). Given the known influences of the hormones on the neuromuscular system, the menopause marks a distinct time within the female lifespan that may exacerbate neuromuscular deconditioning into older age. A greater understanding of this sex-specific alteration may provide more effective interventions and crucially, more appropriate timing of interventions.

Neuromuscular alterations with ageing

Human movement results from actions of motoneuronal output, which are an amalgamation of a multitude of descending systems and afferent inputs. These neural inputs are converted

into mechanical contraction of skeletal muscle, a process that can be enhanced or deteriorated across the spectrum of health and disease. Whilst detailed reviews of the neural structures involved in force generation can be found elsewhere (25), this section intends to provide an overview of motor systems, in order to contextualise the following sections that critically discuss available evidence regarding how biological sex and sex hormones mediate neuromuscular ageing.

Synaptic Input to the Motoneuron Pool

The majority of motor commands are sent from supraspinal centres, namely the motor cortex. Within this brain region, complex networks of inhibitory and excitatory neurons modulate descending drive to the motoneuron (26). Within the context of ageing, the balance between cortical inhibition and excitation is thought to shift, with less inhibition in older groups (27). In the study of Heise *et al.,* the decrease in γ-aminobutyric acid (GABA) mediated inhibition was associated with poorer dexterity, suggesting that ageing impairs the ability of the motor cortex to 'fine-tune' descending motor commands. This age-related disinhibition has been hypothesised to negatively impact the synchronisation of descending impulses from the motor cortex, leading to altered activation of motor units, and impaired control of force (28).

Neurons originating in cortical and subcortical structures form mono- and poly-synaptic connections with motoneurons, known as the descending tracts. These tracts each have specific characteristics that influence their functional roles, and human movement is a collaborative effort between descending and reflex inputs to the motoneuron pool. One classically studied structure is the corticospinal tract, which consists of pyramidal neurons that form mono- and di-synaptic connections with lower motoneurons. Evidence from non-human primates demonstrates how corticospinal neurons alter their firing rate during muscle contraction (29), eventually leading neurophysiologists to label it 'the primary conduit for voluntary motor control'. Research utilising transcranial magnetic stimulation (TMS) as an investigative tool has demonstrated older adults to have smaller evoked responses during

isometric (30) and dynamic (31) contractions of the upper and lower limbs. A smaller evoked response to TMS suggests that excitability of the corticospinal tract is impaired, potentially related to the age-related loss of myelinated corticospinal neurons (32). Recently, Škarabot *et al.* (31) suggested that the age-related loss of corticospinal excitability requires a compensatory increase in volitional drive to maintain force output in elderly individuals. The net result of this alteration in synaptic input to the motoneuron pool is impaired motor unit behaviour and a loss of either force generating capacity or force control.

In addition to corticospinal input, other descending tracts are implicated in the generation and control of force. The reticular formation, a structure of neurons within the brainstem, forms mono- and di-synaptic connections with the motoneuron pool via the reticulospinal tract. The reticulospinal tract was typically considered to be secondary to the corticospinal tract (33), however more recent evidence from non-human primates has suggested that it plays a primary role in generating gross force, whilst the corticospinal tract may be most involved in fine motor tasks (34). Indeed, Tapia *et al.* (35) argue that as the reticular formation receives input from the motor cortex, the cortico-reticulospinal pathway should be considered as important as the corticospinal pathway. Human literature researching the properties of the reticulospinal tract with ageing is sparse, however Maitland and Baker (36) demonstrated smaller ipsilateral motor evoked potentials (iMEP) amplitude, an index of reticulospinal tract excitability in older adults compared to young. In the older group, the size of iMEPs was predictive of grip strength, implying that those with weaker reticulospinal projections were at risk of age-related muscle weakness.

Motor unit discharge is also altered by the reflexive synaptic input which originates in peripheral sensory receptors. For instance, input from muscle spindle Ia afferent neurons provides an excitatory stimulus to the motoneuron pool, which can be measured with evoked responses such as the Hoffman (H) reflex. H reflex amplitudes have been demonstrated to be smaller in elderly groups compared to young (31), implying that the Ia afferent transmission to

the motoneuron pool is impaired with ageing. Collectively, synaptic inputs to the motoneuron play an important role in determining nervous system output (i.e., motor unit behaviour); the amalgamation of multiple sources of neural excitation/inhibition are thereafter converted into a single, repeating signal that is carried to the target muscle(s) via motoneurons to elicit muscle contraction. The ageing process has the potential to alter these inputs and induce functional change (i.e., weakness, unsteadiness, etc).

Motor Unit Discharge Properties

As mentioned above, the motoneuron pool receives input from a multitude of pre-synaptic sources; the motor unit (i.e., a motoneuron and the muscle fibres it innervates) then converts these inputs into muscle contraction via the rate at which action potentials are generated. Evidence from post-mortem anatomical studies demonstrated that older adults have ~30% fewer motor units than younger adults in the lower limbs, which is supported by in vivo electromyographic studies that quantify the motor unit number estimate (MUNE) (37,38). The available data show no sex difference in MUNE (39,40) or associated remodelling (41). Although the progressive loss with age is a key contributor to the loss of muscle mass (42), the function of surviving motoneurons continue to mediate force production and control.

During muscle contractions, force is controlled via the manipulation of the motor unit recruitment and their discharge rate (43). Depending on the speed of contraction, motor unit discharge rates can vary between approximately five to several hundreds of pulses per second (pps). For instance, during rapid contractions Del Vecchio *et al.* (44) demonstrated that peak discharge rates were as high as 227 pps, which was positively associated with the rate of force development. The ability to rapidly increase motor unit discharge rate is attenuated by approximately a third with age (45) and is accompanied by a corresponding decrease in rate of force development. This strong relationship between motor unit discharge rate and functional measures has led researchers to suggest this neurophysiological attribute to be a

key area when considering the risk of falls in elderly individuals. During maximal intensity sustained contractions, the peak discharge rate of motor units is lower compared to rapid contractions, yet still plays an important role in maximal force generating capacity, with Kamen *et al.* (46) showing R^2 values of 0.88 when plotting knee-extensor maximal force against vastus lateralis maximal motor unit discharge rate. The peak discharge rate during sustained contractions is a rather plastic attribute of the nervous system, and in the majority of data this is lower in old compared to young during normalised contraction levels, however this is dependent on the muscle assessed (47).

As well as firing rate, the variability of motor unit firing is also tightly linked to the control of force. Force steadiness (i.e., standard deviation or coefficient of variation of force during a sustained contraction) worsens with age and is an explanatory variable for functional measures such as walking performance, dexterity, and falls risk. It also appears to be tightly linked with the variability of net excitatory synaptic input to the motor unit, with a positive association between the two parameters (48). Accordingly, Castronovo *et al.* (49) observed that older adults' poorer force steadiness was associated with larger amplitudes in the oscillation of the common synaptic input to the motor units.

The relationship between synaptic input and motor unit discharge is non-linear, and this non-linearity is driven by monoaminergic input (i.e., serotonin and noradrenaline), which generates persistent inward currents (PICs) in motoneuron dendrites. The role of PICs within motoneurons is to amplify and prolong synaptic input, with simulations suggesting that synaptic input alone is only able to produce ~40% of maximal force in the absence of PICs (50). Literature concerning estimates of PICs often demonstrates that their magnitude is weakened with age (51–53). This impaired ability to amplify descending commands might therefore be associated with reduced maximal force production and force control in older adults (52).

Ageing elicits changes in all levels of the motor pathway, from the fine-tuning of commands in the motor cortex, to the ability of descending tracts to carry signals to the motoneuronal synapse. The ability of the motor unit to thereafter convert synaptic input into repeated discharges is altered by age, with the net result being functional impairments such as reduced maximal force generating capacity and rate of force development, as well as poorer force steadiness/control. Given the increasingly aged population it is timely that current evidence is utilised to provide targeted directions for research and/or interventions to support improvements in age related changes to the neuromuscular system.

Hormonal influences on the motor cortex and descending tracts

Animal models provide compelling evidence on the effects of oestrogen and progesterone on neural excitability. These studies have illustrated that oestrogen promotes excitability by interacting with the glutamatergic pathway in a dose dependent manner (54). In rat hippocampal neurons, oestrogens induce long-term potentiation (LTP) by promoting the glutamate sensitivity of N-methyl-D-aspartate (NMDA) receptors (55). In contrast, progesterone exerts an inhibitory effect via the gamma-aminobutyric acid (GABA) pathway, but also through suppression of glutamatergic excitatory activity (56). Collectively, these studies clearly demonstrate the opposing effects of the key female sex hormones on neural excitability.

Testosterone also has the capacity to influence neural function, with in vitro evidence for DHEA having excitatory effects (57), influencing GABA and glutamate release. It seems DHEA and DHEAS target a number of neural receptors, which in turn modulate a number of neurotransmitter systems (58). Testosterone likely exerts some influence on central nervous system function due to the localisation of androgen receptors (AR) within neurons in the brain and spinal cord of rats (59). However, testosterone's role in regulating neural excitability in humans is less clear than that of oestrogen and progesterone.

Human models of neural excitability

A consistent finding in the neurophysiological literature is that there is no change in the size of unconditioned MEPs evoked by TMS across the menstrual cycle (60,61). This would indicate that net excitability of the corticospinal tract remains constant despite hormonal changes across the menstrual cycle. However, as the cellular and animal model work demonstrate, the effects of these hormones are primarily exerted via the main neurotransmitters within the brain, rather than altering descending tract excitability (62). In eumenorrheic women, increased progesterone concentrations increased intracortical inhibition, of the upper (63) and lower limbs (61). Notably, these effects on short intracortical inhibition are absent in women using exogenous hormones (i.e., a contraceptive pill) that minimises fluctuations in sex hormones (61). Progesterone is thought to potentiate GABAergic synapses through the activation of the $GABA_A$ receptor (23), with the relative change in intracortical inhibition across the menstrual cycle comparable to the change induced by GABAergic pharmacological agents (64).

To gain a more detailed insight on the effects of oestrogen on neurotransmission within the motor cortex, Smith *et al.* (65) assessed responses in early and late stages of the follicular phase. It was observed that intracortical facilitation (ICF) was greater in correspondence with increased oestrogen concentrations. ICF is likely mediated by glutamatergic transmission via NMDA receptors, supporting previous animal studies (54,66). Given the well-established changes in the hormonal milieu across the lifespan and the potent effect of sex hormones on neural excitability in young women, there is potential for an age effect, which might be influenced by different stages of the menopause whereby there are dramatic alterations to levels of sex hormones, but this remains relatively unstudied at present.

As well as influencing acute properties of intracortical neurons, sex hormones also mediate models of cortical neuroplasticity. In female rats, long-term potentiation (LTP) is enhanced during the proestrus (high oestrogen) phase compared to low oestrogen states (67).

Comparable human data demonstrates that, in females, when oestrogen is low during the early follicular phase there is a blunted response to repetitive rTMS; thereafter, in the late follicular phase, when oestrogen concentrations are elevated, the facilitatory response to rTMS is restored, and comparable to males (60). Once more, this evidence utilises the model of the eumenorrheic menstrual cycle in young women to determine hormonal influences, however preliminary evidence does exist studying the age/sex interaction across the lifespan. The potential impact of the sex hormones on sex-specific ageing effects was evident following a paired associative stimulation (PAS) protocol, whereby older females did not experience a facilitatory response, but no age-related blunting of the response was apparent in males (68). Polimanti and colleagues (69) also observed a blunted PAS response in aged females that was not evident in aged males. Authors have postulated that the female specific responses could be due to the change in endogenous hormones through the menopause, yet no reproductive information (i.e., menopausal status) regarding female participants was reported.

Further evidence for the influence of sex hormones on neural excitability can also be found within sensory circuits. Hoffman et al., (70) observed a decrease in pre-synaptic inhibition of the H-reflex when concentrations of oestrogen were elevated. Here, the oestrogen-mediated reduction in GABAergic inhibition mirrored that observed in the motor cortex (61,63,65). Taken together, the evidence presented herein demonstrates that changes in the hormonal milieu, at all stages of life, have the capacity to impact on the modulation neural excitability at various levels, which may alter synaptic input to the motoneuron pool. Specifically, the drastic decrease in oestrogen and progesterone across the menopause might accelerate age-related changes in females, compared to the more gradually decrease in testosterone in males. However, there is a relative dearth of studies directly investigating this topic.

Hormonal influences on motor unit behaviour

The sex hormones discussed herein, and mentioned earlier, exert genomic and non-genomic effects on multiple physiological systems including the function *and* structure of motor units.

The extent to which they differentially influence male and female neuromuscular function via these means with respect to sex-specific hormone concentrations is not clearly defined, but it does present an attractive line of research with clear translational relevance.

Males generally possess a greater muscle strength in both the upper and lower extremities, the majority of which can be explained by differences in muscle mass and composition (71), largely influenced by diverse hormonal profiles during adolescence (21). Conversely, females are generally more resistant to neuromuscular fatigue when assessed at normalised forces and across multiple exercise modalities (72). Although not a direct comparison of hormone levels and their effects, a number of studies have highlighted sex differences at the motor unit (MU level) in young people. In the vastus lateralis (VL), females had a higher motor unit firing rate (MUFR) than males at normalised sustained force levels (10 and 25% of max), however the relative change between low and mid-level contractions did not differ, suggesting similar strategies are employed to increase muscle force (73). This greater discharge rate in young females is reported elsewhere in other muscles (74), but is not consistent and is likely influenced by contraction type and intensity (74). A probable mechanistic candidate here is the sex-specific levels of monoaminergic input to motoneurons, and although not directly quantifiable in humans, the magnitude of PIC influences motor unit discharge hysteresis, which can be estimated in humans via the well-established paired MU technique. Of the only study to investigate this in humans, estimates of PIC magnitude were higher in female tibialis anterior (TA), medial gastrocnemius and soleus (75) and although hormone levels were not quantified here, the authors suggested the influence of oestrogen on serotonergic signaling was partly responsible, which has strong mechanistic plausibility.

The naturally fluctuating hormone levels across the eumenorrheic menstrual cycle presents an attractive model to investigate the influence of hormones on MU behavior. This is somewhat impeded by the largely unknown level of intra-individual variability in hormone quantities which may partly explain conflicting findings across basic measures of function (76).

More detailed examinations across the menstrual cycle have been performed using intramuscular EMG, and differences were noted in the initial discharge rate of VL MU across 5 stages of the menstrual cycle, as assessed by body temperature (77). A more recent study with quantification of hormone concentrations and cycle mapping highlighted no differences in functional performance across 3 time points of the cycle (early follicular, ovulation and mid-luteal phases), but did observe a reduced firing rate (MUFR) of low threshold VL MUs in the latter two phases of the cycle, coinciding with environments of increased levels of progesterone (78). Although not conclusive, this suggests a probable neuroinhibitory effect of progesterone impacting early recruited motor units only.

The sex-based differences in VL MUFR at normalised forces observed in young were also observed in older males and females (52). The recruitment strategy, defined as the relative change in MUFR and size of potential when moving from a low to a mid-level contraction, also did not differ between older males and females, as observed in younger counterparts (52,73). However, the sex discrepancy is clearly influenced by age, with profound differences in strength in older individuals, and a greater FR variability and poorer force control of the knee extensors observed in older females compared to older males, a feature not noted in younger counterparts. Although clear mechanisms are not readily available, the post-menopausal hormonal milieu may account for the widening sex differences with advancing age, exacerbating age-related cortical excitation-inhibition imbalances. In a separate study of highly active masters athletes MUFR declined from middle to older age in females, but stayed similar in males, again supporting divergent responses (41). These differences may not be mediated entirely by female hormones and may reflect hormonal influences in ageing males, or a combination of the two. DHEA is commonly higher in males than females and plasma DHEA concentrations were positively associated with MUFR in older active and inactive males (79), and DHEAS was positively associated with compound muscle action potential size in frail older men (80), both of which may reflect neuroprotective effects. Given the differences in the hormonal environments of the adult lifespan between males and females, it is probable such

distinction influences the age-related changes to motor unit function, and possibly structure, contributing to the sex-health paradox in older age.

Motor units in aged skeletal muscle may be generally described as being fewer in number and larger in size due to their progressive loss and subsequent remodeling (38,81), manifesting as an expansion of MU size (innervation ratio) to compensate for declining numbers and to minimise fibre loss (82). Thus, structural alteration of MUs via axonal sprouting and NMJ re-/formation is possible, and animal model and cell culture evidence highlight hormones as a positive promoter of this process. Motoneuron dendrite atrophy in response to castration was reversed by exogenous testosterone administration in adult rats (83), and in female rats with spinal cord injury, exogenous testosterone attenuated atrophy of dendrites (84). Similarly, estradiol reduced spinal-cord injury induced lesion volume in female rats (85). Although experiments of an equivalent detail are not replicable in humans, anabolic hormone concentrations were strongly associated with electrophysiological measures of skeletal muscle in pre-frail and frail older men (80). Advances in needle EMG techniques combined with histological markers of muscle fibre denervation have the potential to reveal structural adaptation of MUs in a minimally invasive approach in humans.

The wider implications

Frailty and falls risk

Understanding sex differences in neuromuscular ageing is imperative to addressing sex differences across the spectrum of health and disease. The number of people aged over 60 years in the UK is growing more rapidly than any other age group. In 2017 the number of over 85s was 1.35 million, in two years this number is set to reach around 1.54 million (a rise of 14%) and by 2031 projected to rise even further, reaching 2.01 million (86). It is well established that older females have a greater frailty index and risk of falls than male counterparts (87). Whilst frailty and falls risk are multi-factorial that have complex physiological, psychological and sociological determinants, this review hypothesises that the interaction

between ageing and hormonal changes across the lifespan has the potential to influence key attributes of the neuromuscular system that are implicated in quality of life and independence. Identifying how sex hormones influence synaptic input to the motoneuron pool and motor unit discharge properties differently in males and females will enable researchers to highlight physiological targets for therapeutic interventions aiming to attenuate neuromuscular impairment(s) related to poor health and disease.

Sex discrepancies in neurorehabilitation

Comparative to males, females have a poorer quality of life, mobility, and functional outcomes after neurological diseases such as stroke, even after accounting for socioeconomic, clinical, and stroke-specific factors. Intensive physiotherapy, the 'gold standard' recovery intervention, leads to a three-times greater probability of achieving functional independence in males compared to females (88). Research into the contributing factors to this sex difference frequently neglect the hormonal status of females. Given that 38% of people suffering strokes are middle aged and within the typical age range for the menopause (40-69 years), further mechanistic and clinical investigation on this topic is warranted.

Nervous system 'responsiveness' to interventions (neuroplasticity) can be experimentally probed by techniques such as non-invasive neurostimulation, with the response to protocols such as paired associative stimulation (PAS) changing in parallel with motor recovery in stroke patients (89). As outlined in the preceding sections, the response to PAS appears to be blunted in elderly females, whereas the male response is maintained (68). If the dramatic decrease in sex hormones across the menopause is implicated in blunting neuroplasticity, this could provide a mechanistic explanation for the poorer functional outcomes after stroke and further adds reasons for these hormonal changes to become a therapeutic target for improvements in neuromuscular function with ageing.

Future Directions

A recent audit of journals within the field of physiology and sports science highlighted an underrepresentation of female participants (90). However, it was noted that 63% of publications did include both sexes, yet, of these, few reported sex-specific results. Therefore, in addition to including more females in studies investigating neuromuscular ageing, and given the evidence provided herein we believe the following points should also be addressed in future research design and data collection.

Disaggregating data by the biological sex of participants is a simple way to begin addressing this problem (91,92). For instance, when male and female participants are pooled together to form a 'young' or an 'old' group, it is a missed opportunity for exploring potential sex differences in ageing. Indeed, the authors themselves admit to missing this opportunity in previous research (31). As acknowledged by other articles on this topic (91,92), there are additional considerations that must be accounted for when performing these analyses. If a study is adequately powered to answer its primary research question (i.e., the effect of ageing on an outcome variable), it does not necessarily mean that it is adequately powered to answer the question of how sex mediates the ageing process. Therefore, any potential sex differences that are inferred from this approach must be robustly followed up with experimental designs that are statistically powered to confirm or reject the initial conclusions.

The National Institute for Health and Care Excellence (NICE) guidance for diagnosis and management of menopause (93) provides a toolkit for researchers to categorise female participants by menopausal status without requiring quantification of hormone concentrations or further laboratory testing. Information regarding vasomotor symptoms and menstrual disturbances can be obtained from participants' self-reporting in questionnaires and used to define menopausal status. Researchers with the capabilities to do so might wish to verify this self-reporting of menopausal status with hormonal quantification. However, given the limitation on funding availability for such studies, as a first step, the NICE guidelines provide a method

of categorising participants specific to their menstrual status to allow exploration of novel research questions.

Menopausal status is one of many reproductive variables that might influence nervous system properties. Additionally, the use of exogenous hormones through hormonal contraceptives and hormone replacement therapy has the potential to alter neuromuscular function but is a drastically underexplored area. As Pletzer & Kerschbaum highlighted in their paper titled: *"50 years of hormonal contraception—time to find out, what it does to our brain"*, far less is known about the neuroactivity of synthetic hormones compared to their endogenous counterparts (94). As mentioned previously pregnancy induces considerable changes to the female hormonal profile and therefore instigating both short and long-term changes within the nervous system (95). Collectively, this evidence implies that researchers should consider compiling a comprehensive profile of reproductive history for female participants. Tools such as the Endometriosis Phenome and Harmonisation Project (EPHect) questionnaire are, by nature, specific to one of the aforementioned influences, yet a subset of such questions could be utilised to capture a well-rounded understanding of the female reproductive history.

Summary

The available evidence from a variety of experimental models exploring the effects of sex hormones on nervous system properties highlights oestrogen's ability to potentiate the responsiveness of neurons to excitatory glutamatergic input, whereas progesterone and its metabolites exert inhibitory influences via the $GABA_A$ receptor. In humans, these data are mirrored in responses to paired-pulse TMS, which reveal changes in the excitatory/inhibitory balance across the eumenorrheic menstrual cycle (61). Likewise, the menstrual cycle has been used to demonstrate the variation in motor unit discharge properties exhibited in differing hormonal environments (78).

Whilst these studies of neurophysiology in *ex vivo* neuronal circuits, rodents, and across the human menstrual cycle reveal the effects of sex hormones on nervous system properties, their translation to an ageing model could be questioned. Fluctuations in sex hormones across the eumenorrheic menstrual cycle are transient and cyclical, whereas the menopause is a permanent termination in ovulation and menstrual cycles. Caution must therefore be urged when attempting to make conclusions about the influence of these hormones in neuromuscular ageing from experimental models based around the menstrual cycle and comparisons between young males and females. Indeed, the present review highlights the lack of literature that uses the menopause as a model for investigating sex hormone effects, which is a concern considering the greater societal burden of age-related conditions in females compared to males.

Preliminary evidence suggests that females might experience blunted responses to non-invasive neurostimulation in older age, and females might experience a greater age-related decline in motor unit discharge rates. These findings could provide mechanistic insight into sex discrepancies within the fields of frailty and neurorehabilitation, however, it is imperative that further research interrogating the ageing motor pathway in both sexes is required. Such research must take into consideration the menopausal transition as a critical period within the hormonal lifespan that may significantly contribute to the differences between males and females and the ageing neuromuscular system.

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