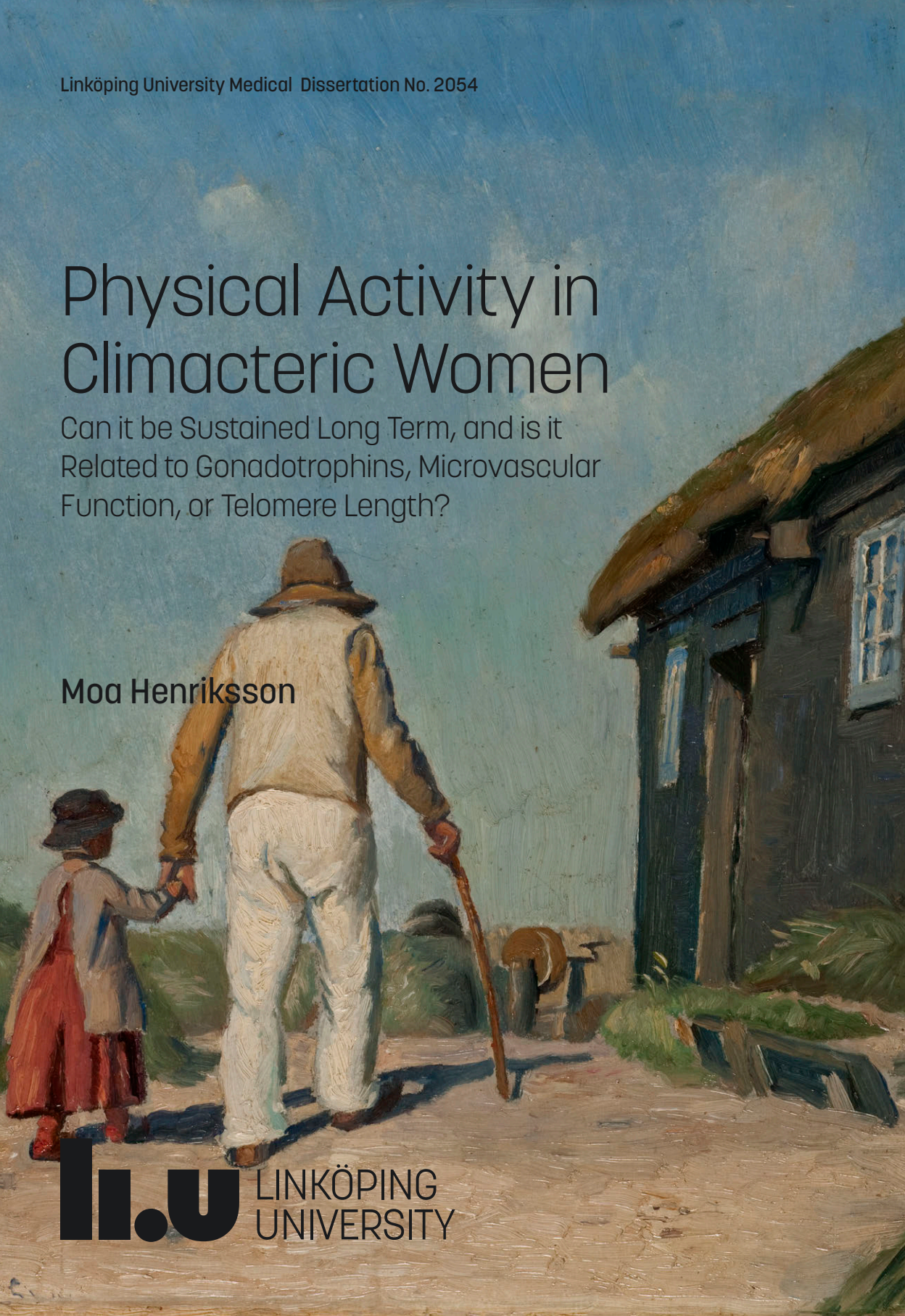
Linköping University Medical Dissertation No. 2054

Physical Activity in Climacteric Women

Can it be Sustained Long Term, and is it Related to Gonadotrophins, Microvascular Function, or Telomere Length?

Moa Henriksson



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Physical Activity in Climacteric Women:

Can it be Sustained Long Term, and is it Related to Gonadotrophins, Microvascular Function, or Telomere Length?

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Cover image: Michael Ancher: Blind Christian. Study, c. 1881, Nationalmuseum (Photo: Per-Åke Persson), public domain.

ABBREVIATIONS

BMI	body mass index
CI	confidence interval
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
DRS	diffuse reflectance spectroscopy
EDTA	ethylenediaminetetraacetic acid
EPOS	Enhanced Perfusion and Oxygen Saturation
FSH	follicle-stimulating hormone
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GnRH	gonadotrophin-releasing hormone
HbA1c	glycated haemoglobin
IPAQ	International Physical Activity Questionnaire
ITT	intention to treat
KNDy	kisspeptin/neurokinin B/dynorphin
LDF	laser doppler flowmetry
LH	luteinising hormone
LTL	leucocyte telomere length
MET	metabolic equivalent of task
MHT	menopausal hormone therapy
MVPA	moderate-to-vigorous physical activity
mRNA	messenger ribonucleic acid
NK3R	neurokinin 3 receptor

OxyP	post-occlusive peak oxygen saturation
POA	preoptic area
PP	per protocol
qPCR	quantitative polymerase chain reaction
RCT	randomised controlled trial
RM	repetition maximum
RNA	ribonucleic acid
SCAPIS	Swedish CArdioPulmonary bioImage Study
SD	standard deviation
TACR3	tachykinin receptor 3
VMS	vasomotor symptoms

ABSTRACT

Background: The menopausal transition is accompanied by vasomotor symptoms (VMS) and occurs during a period of increasing cardiovascular risk and biological ageing. Physical activity may alleviate symptoms and promote health. However, whether increased physical activity can be sustained long term remains uncertain. Its neuroendocrine, microvascular, and cellular correlates are also incompletely understood.

Aim: To investigate the role of physical activity in climacteric women, with a particular focus on whether increased physical activity can be sustained long term, circulating gonadotrophin concentrations, cutaneous microvascular reactivity, and leukocyte telomere length (LTL) as a marker of cellular ageing.

Methods: The thesis combined an open randomised controlled trial with a population-based cross-sectional study. The STYRKA trial included 65 postmenopausal women with VMS and low physical activity. Participants were randomised to 15 weeks of resistance training or an untreated control condition. Physical activity was assessed by questionnaire over 2 years. Plasma luteinising hormone (LH) and follicle-stimulating hormone (FSH) were measured at baseline and 15 weeks. LTL was measured by quantitative polymerase chain reaction at baseline, 15 weeks, and 2 years. We also examined whether physical activity during follow-up was related to LTL change. The cross-sectional analyses included 1072 women aged 50–64 years from the Swedish CArdioPulmonary bioImage Study. Cutaneous microvascular reactivity was assessed as peak oxygen saturation after release of arterial occlusion (OxyP). Menopausal status, menopausal hormone therapy (MHT) use, and VMS were self-reported. Physical activity and sedentary time were assessed by accelerometry.

Results: Self-reported physical activity increased and remained above baseline after 2 years in both original randomised groups. The control group increased its activity after being offered an introduction to resistance training and temporary gym access following the initial intervention period. Among participants meeting the prespecified compliance criterion, LH but not FSH

decreased compared with the control group. Changes in gonadotrophins were not associated with changes in VMS. OxyP differed across tertiles of accelerometer-measured moderate-to-vigorous physical activity. OxyP was higher in the intermediate and highest tertiles than in the lowest tertile, whereas the two higher tertiles did not differ. OxyP did not differ across sedentary time tertiles or by menopausal status, current MHT use, or VMS frequency. LTL change did not differ significantly between the original randomisation groups at 15 weeks or 2 years. Self-reported physical activity during follow-up was not significantly associated with LTL change from 15 weeks to 2 years.

Conclusions: Increased self-reported physical activity was sustained for 2 years after resistance training and behavioural support. The reduction in LH among participants meeting the compliance criterion suggests that resistance training may influence neuroendocrine regulation. Women in the two higher tertiles of habitual moderate-to-vigorous physical activity had greater cutaneous microvascular reactivity. Neither resistance training nor follow-up physical activity showed a clear relation to LTL change, although estimates were imprecise. Overall, the findings support resistance training with behavioural support as a feasible approach to promoting sustained physical activity in climacteric women. They also identify promising neuroendocrine and microvascular findings that warrant further investigation.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Klimakteriet är den period då äggstockarnas hormonproduktion minskar och menstruationerna så småningom upphör. Den sista menstruationen kallas menopaus. Omkring åtta av tio kvinnor får värmevallningar eller nattliga svettningar under klimakteriet. För vissa är besvären lindriga, medan andra får störd sömn och försämrad livskvalitet. Minskade östrogennivåer påverkar hjärnans reglering av kroppstemperaturen och bidrar till att värmevallningar uppstår. Hormonbehandling kan lindra symtomen, men passar inte alla kvinnor. Det behövs därför också andra sätt att stödja hälsa och välbefinnande under denna del av livet.

Fysisk aktivitet har välkända hälsofördelar, bland annat för muskler, skelett och hjärt–kärlsystem. Samtidigt återstår frågor om vilken roll fysisk aktivitet har under klimakteriet. Avhandlingen bygger på en styrketräningsstudie och en delstudie inom den stora svenska befolkningsstudien SCAPIS. Följande frågor stod i fokus:

Kan styrketräning och stöd hjälpa kvinnor med låg fysisk aktivitet att bli mer aktiva och behålla en högre aktivitetsnivå över tid? Kan styrketräning påverka hormonregleringen och ge ledtrådar till hur träning kan lindra värmevallningar? Skiljer sig funktionen i hudens små blodkärl mellan kvinnor med olika nivåer av fysisk aktivitet? Kan styrketräning påverka telomerlängden, som är kopplad till cellernas åldrande?

I träningsstudien deltog 65 kvinnor som hade passerat menopaus, besvärades av värmevallningar och hade låg fysisk aktivitet. Kvinnorna lottades till styrketräning tre gånger i veckan under 15 veckor eller till en kontrollgrupp. Deltagarna fick individuella instruktioner av en fysioterapeut, som också handledde ett träningspass i veckan. Efter de första 15 veckorna erbjöds kontrollgruppen en introduktion till styrketräning och fyra månaders gymkort. Kvinnornas fysiska aktivitet följdes med frågeformulär under två år. Blodprover användes för att undersöka hormonnivåer och telomerlängd.

Kvinnorna som lottades till styrketräning ökade sin fysiska aktivitet under träningsperioden. Efter två år var den självrapporterade aktivitetsnivån fortfarande högre än före studiens början. Även kontrollgruppen ökade sin

aktivitet efter att ha erbjudits stöd för att komma igång med träningen. Vid tvåårsuppföljningen fanns ingen tydlig skillnad mellan grupperna. Resultaten visar att en ökad aktivitetsnivå kunde bestå över tid i denna grupp av kvinnor. De talar också för att en introduktion till styrketräning, tillgång till träningslokaler och stöd kan hjälpa kvinnor att etablera mer varaktiga aktivitetsvanor. Det går däremot inte att avgöra vilken del av stödet som hade störst betydelse.

Tidigare resultat från samma träningsstudie visade att styrketräning minskade antalet värmevallningar. En gemensam länk mellan värmevallningar och hormonreglering är en grupp nervceller i hjärnan som kallas KNDy-neuron. Dessa deltar både i regleringen av kroppstemperaturen och i styrningen av hormonfrisättning. För att undersöka om träningen påverkade hormonregleringen mättes luteiniserande hormon (LH) och follikelstimulerande hormon (FSH) i blodet. Dessa två hormoner bildas i hypofysen, reglerar äggstockarnas funktion och stiger i blodet efter menopaus.

Bland kvinnorna som uppfyllde studiens förutbestämda krav på träningsmängd minskade LH mer än i kontrollgruppen. Skillnaden för FSH var inte statistiskt säkerställd. När samtliga kvinnor analyserades utifrån den grupp de hade lottats till sågs ingen säkerställd skillnad för något av hormonerna. LH-minskningen är förenlig med hypotesen att styrketräning kan påverka KNDy-neuronens aktivitet och därigenom både hormonfrisättning och värmevallningar. Nervcellernas aktivitet mättes dock inte direkt, och något samband mellan förändringarna i LH och värmevallningar kunde inte visas. Fyndet ger därför anledning att undersöka denna möjliga mekanism vidare.

I delstudien inom SCAPIS deltog 1 072 kvinnor i åldern 50–64 år från Linköping. Mikrocirkulationen består av de små blodkärl som förser vävnaderna med syre och näring. Kärlens reaktion bedömdes genom att mäta blodets högsta syremättnad i huden efter att blodflödet tillfälligt hade stoppats med en blodtrycksmanschett och sedan återställts. Fysisk aktivitet och stillasittande registrerades med en rörelsemätare.

Kvinnorna i grupperna med medelhög och hög nivå av fysisk aktivitet av minst måttlig intensitet hade högre maximal syremättnad än kvinnorna i den minst aktiva gruppen. Skillnaderna kvarstod när analyserna tog hänsyn till bland annat ålder, BMI, rökning och diabetes. Fyndet talar för att fysisk aktivitet kan ha betydelse för hudens mikrocirkulation och motiverar fortsatta studier av om träning kan förbättra de små blodkärlens reaktion när blodflödet återställs.

Däremot sågs inga tydliga skillnader mellan grupper med olika mängd stillasittande. Kärlreaktionen skilde sig inte heller mellan kvinnor före och efter menopaus som aldrig hade använt hormonbehandling. Bland kvinnor efter menopaus sågs inga skillnader mellan dem som använde hormonbehandling och dem som aldrig hade gjort det, eller mellan grupper med olika frekvens av värmevallningar. Resultaten gav alltså inget stöd för att dessa klimakteriерelaterade faktorer hade betydelse för just den kärlreaktion som mättes.

Avhandlingen undersökte även telomerer, skyddande delar av arvsmassan i ändarna av kromosomerna. Telomerer förkortas vanligtvis när celler delar sig och är kopplade till cellernas åldrande. I träningsstudien mättes telomerlängden i vita blodkroppar. Förändringen i telomerlängd skilde sig inte statistiskt säkerställt mellan de ursprungliga grupperna efter 15 veckor eller två år. Inte heller sågs något tydligt samband mellan fysisk aktivitet under uppföljningen och förändrad telomerlängd. Resultaten gav inget tydligt stöd för att träningen påverkade telomerlängden, men få deltagare och osäkra skattningar begränsar slutsatserna.

Avhandlingens viktigaste resultat är att kvinnor med värmevallningar och låg fysisk aktivitet kunde öka sin aktivitet och behålla ökningen under två år. Styrketräning tillsammans med stöd framstår därför som en möjlig väg till mer varaktig fysisk aktivitet under klimakteriet. Resultaten om LH och hudens mikrocirkulation ger samtidigt nya utgångspunkter för forskning om kroppens svar på träning. Fortsatta studier behövs för att klarlägga mekanismerna och avgöra vilken betydelse dessa fynd har för kvinnors långsiktiga hälsa.

LIST OF SCIENTIFIC PAPERS

I. Increased and sustained physical activity in postmenopausal women: 2-year follow-up of a resistance training intervention.

Henriksson M, Nilsson S, Berin E, Hammar M, Spetz Holm AC, Lindblom H. Maturitas. 2025; 202:108 696.

II. Resistance training reduced luteinising hormone levels in postmenopausal women in a substudy of a randomised controlled trial: A clue to how resistance training reduced vasomotor symptoms

Nilsson S, * Henriksson M, * Berin E, Engblom D, Spetz Holm AC, Hammar M. PLoS One. 2022;17(5).

III. Microvascular reactivity in mid-life women: Influence of menopause, hormone therapy, vasomotor symptoms, physical activity, and sedentary behaviour.

Henriksson M, Berin E, Hammar M, Jonasson H, Bergstrand S, Strömberg T, Östgren CJ, Leander K, Spetz Holm AC.

Manuscript.

IV. Leukocyte telomere length following resistance training in postmenopausal women: An open randomised controlled trial with a 2-year extension.

Henriksson M, Spetz Holm AC, Hammar M, Asghar M.

Manuscript.

TABLE OF CONTENTS

Abbreviations ..... **iv**
Abstract ..... **vi**
Populärvetenskaplig sammanfattning ..... **viii**
List of scientific papers ..... **xi**
Background **1**
Menopause and the climacteric 1
Mechanisms underlying vasomotor symptoms 5
Cardiovascular function and the microcirculation 12
Ageing and telomeres 16
Physical activity 19
Thesis rationale **25**
Hypothesis **26**
General hypothesis 26
Specific hypotheses 26
Aims **27**
General aim 27
Specific aims 27
Material and methods **28**
Study overview 28
Study designs and populations 29
Exposures 31
Outcomes and outcome assessment 32
Covariates 36
Statistical analyses 36
Ethical approvals and considerations 39
Results and comments **41**
Sustained physical activity 41
Gonadotrophin concentrations 44
Microvascular function 46
Leukocyte telomere length 50
Discussion **53**
Methodological considerations 53
Findings in relation to the thesis objectives 60
Future perspectives 63
Acknowledgements **65**
References **67**
Appendix **85**
Supplementary questionnaire on women's health in SCAPIS 85
Papers I-IV

BACKGROUND

Menopause and the climacteric

Menopause, defined as the final menstrual period, represents the permanent cessation of ovarian follicular activity and reproductive function. The diagnosis is established after 12 months of amenorrhea in the absence of other causes [1]. The mean age at natural menopause is approximately 51 years, although substantial interindividual variation exists [2]. About 5% of women have early menopause and about 2.5% have late menopause, i.e. at or after 56 years [3]. Smoking has consistently been associated with earlier menopause and has been reported to advance menopause by approximately 1-2 years [4]. The term climacteric describes the broader period during which women transition from reproductive to postmenopausal life, including the years surrounding menopause. The word climacteric originates from the Greek word *klimakter*, meaning "ladder" or "critical step", reflecting the concept of transition from one stage of life to another.

Endocrine Changes during the Menopausal Transition

Female reproductive ageing is closely linked to the gradual depletion of the ovarian follicular reserve. The number of ovarian follicles declines progressively throughout life, beginning during foetal development continuing throughout childhood, during use of contraceptives, pregnancy, and lactation. This decline ultimately results in loss of ovarian endocrine and reproductive function when the remaining follicular pool falls below approximately 1000 follicles regardless of age [5]. Menopause therefore reflects depletion of the follicular reserve to a critical threshold rather than chronological ageing itself.

During the reproductive years, ovarian hormone production is regulated through the hypothalamic-pituitary-ovarian axis (Figure 1). Gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates pituitary secretion of follicle-stimulating hormone (FSH) and luteinising hormone (LH), which in turn regulate follicular development and ovarian hormone production [6]. Oestradiol and inhibin produced by granulosa cells exert negative feedback on pituitary gonadotropin secretion and thereby contribute to the regulation of menstrual cyclicity [7]. Oestrogen feedback on GnRH

secretion is mediated through kisspeptin/neurokinin B/dynorphin (KNDy) neurons located in the arcuate nucleus of the hypothalamus [8]. Oestradiol suppresses both LH and FSH secretion, whereas inhibin suppresses FSH specifically [9].

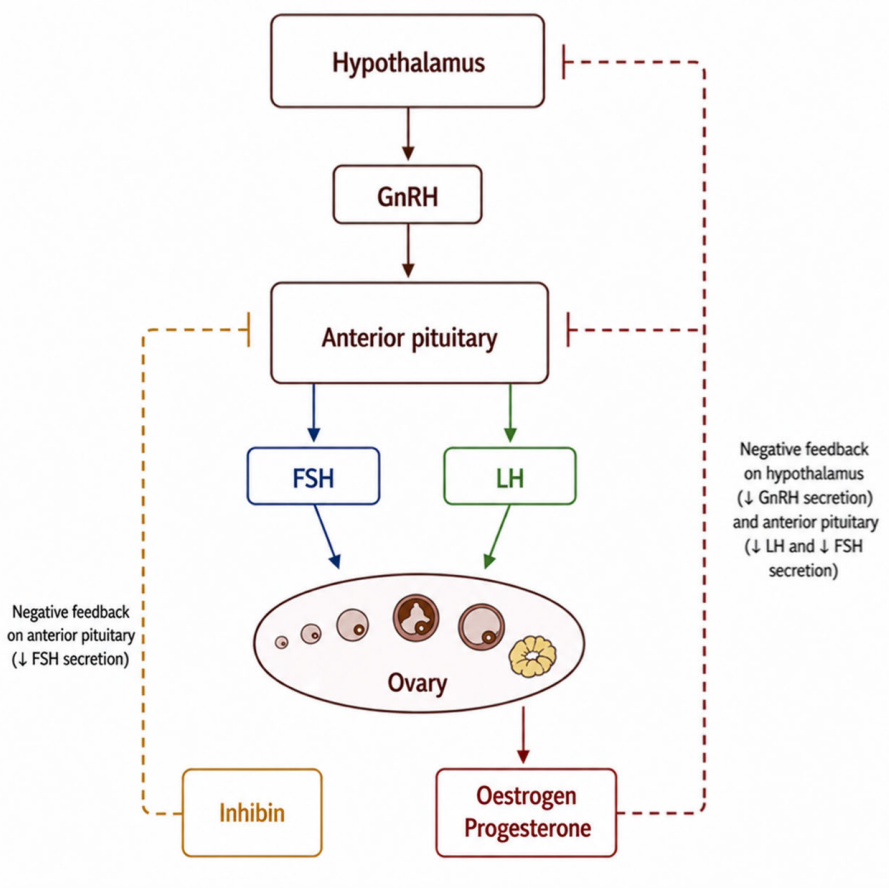

Figure 1. The hypothalamic-pituitary-ovarian axis

Schematic illustration of the hypothalamic-pituitary-ovarian axis and its endocrine feedback mechanisms.

The years preceding menopause are characterised by progressive alterations in menstrual cyclicity. Menstrual irregularity is the most objective clinical marker of the approaching menopausal transition and may present as shorter ovulatory cycles or more frequent anovulatory cycles [10-12]. Longitudinal

studies have shown that most women experience several years of increasing menstrual irregularity before menopause, whereas abrupt cessation of menses without a preceding period of irregularity occurs in about 10% of women [13]. For most women, the menopausal transition lasts approximately 2-8 years from onset of menstrual irregularity to menopause [13].

The endocrine alterations associated with reproductive ageing primarily reflect declining ovarian follicular reserve and reduced inhibin secretion from granulosa cells [14]. Since inhibin exerts negative feedback on pituitary FSH secretion, declining inhibin levels contribute to progressively increasing circulating FSH concentrations during menopausal transition and after menopause [9]. The inability of exogenous oestrogen to normalize postmenopausal FSH levels highlights the important role of inhibin in pituitary FSH suppression. LH levels generally remain relatively stable during the earlier phases of the menopausal transition before increasing more substantially after menopause [15]. Following menopause circulating gonadotropin concentrations increase, with FSH rising more prominently (approximately 10-20-fold) than LH levels (approximately 3-fold) [16]. The more pronounced increase in FSH is likely related to both its longer circulating half-life compared with LH and the absence of inhibin-mediated negative feedback on LH secretion. The characteristic changes in circulating FSH, LH, and oestrogen concentrations across the menopausal transition are illustrated in Figure 2.

During the early menopausal transition, oestradiol concentrations may increase slightly in response to increasing FSH [17] or remain relatively preserved or fluctuate. However, with progressive follicular depletion, circulating oestrogen concentrations decline markedly, beginning approximately two years prior to menopause [18]. Once the ovarian follicular reserve is completely depleted, the ovaries no longer produce oestrogen. Before menopause, circulating oestradiol concentrations vary markedly across the menopausal transition, ranging from approximately 150-1470 pmol/L. After menopause, concentrations are typically 40-70 pmol/L [19, 20]. Remaining oestrogen production depends on the peripheral conversion of androgens of adrenal and ovarian stromal origin, primarily androstenedione and testosterone, to estrone and oestradiol, respectively [20, 21]. Testosterone

concentrations are relatively stable during the menopausal transition [20] and hence late postmenopause may be described as a state of relative androgen excess as the androgen/oestrogen ratio increases drastically.

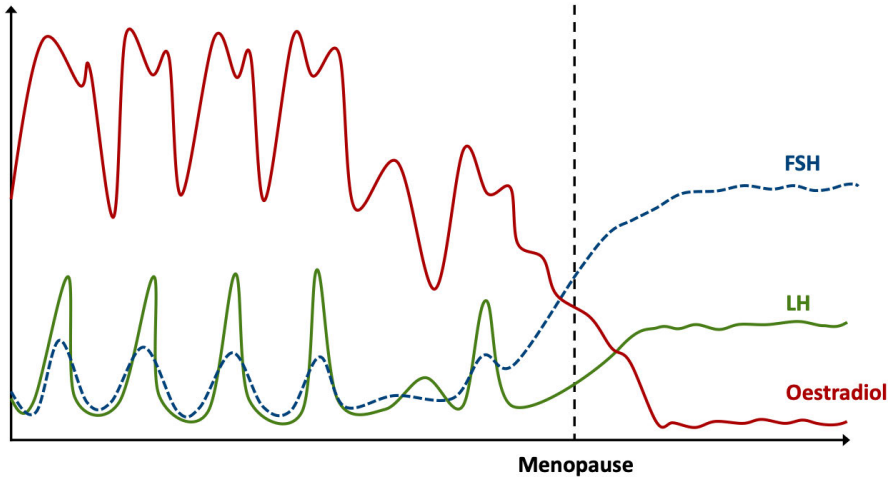

Figure 2. Hormone changes during menopausal transition

Schematic representation of hormonal changes before, surrounding, and after menopause. Hormone concentrations and fluctuations are simplified and not shown to scale.

Symptoms due to menopausal transition

Progressive loss of ovarian follicular function and the subsequent decline in oestrogen production during the climacteric give rise to several characteristic symptoms and physiological alterations, including:

1. Menstrual disturbances with altered cycle length, increasingly occurring anovulatory cycles, and eventually amenorrhea
2. Hot flushes and night sweats, together referred to as vasomotor symptoms (VMS)
3. Genitourinary syndrome of menopause with vulvovaginal atrophy and atrophy of urethral and urinary bladder epithelium
4. Long-term consequences of oestrogen deficiency, including increased risk of osteoporosis and cardiovascular disease (CVD)

Other symptoms frequently reported during the menopausal transition include sleep disturbance, subjective memory or concentration difficulties, palpitations, and muscle and joint pain. However, these symptoms are non-specific, may have causes unrelated to menopause, and cannot be attributed to the menopausal transition with the same certainty as the manifestations listed above.

Vasomotor symptoms

VMS, consisting of hot flashes and night sweats, are the hallmark symptoms of the climacteric. Hot flashes are typically experienced as transient episodes of heat sensation with a duration between seconds to several minutes involving the face, neck, and upper body, and may be accompanied by cutaneous flushing and/or sweating, sometimes followed by chills [2]. Night sweats are generally regarded as hot flashes occurring during sleep [22]. VMS affect up to 80% of women [2].

The median duration of VMS is about seven years, with earlier symptom onset associated with longer persistence of symptoms [23]. The severity and frequency on VMS vary substantially between women, ranging from occasional mild symptoms to frequent symptoms that markedly impair sleep, occupational functioning, and quality of life [2]. VMS peak during the first year after menopause and thereafter diminish over time [24]. However, VMS have been reported in women up to 85 years of age [25].

Mechanisms underlying vasomotor symptoms

Thermoregulatory dysfunction due to declining oestrogens

Current evidence suggests that oestrogen withdrawal disrupts hypothalamic thermoregulatory pathways. VMS are believed to arise from activation of heat dissipation responses triggered by relatively small elevations in core body temperature. In asymptomatic individuals, a thermoneutral zone of approximately 0.4°C is maintained. Within this range, fluctuations in core body temperature are regulated by subtle and largely imperceptible mechanisms without triggering overt thermoregulatory responses such as flushing or sweating. In postmenopausal women with VMS, the thermoneutral zone is narrowed, such that even minor increases in core body

temperature may exceed its limits and activate compensatory heat loss responses including cutaneous vasodilation and activation of cholinergic sweat glands, resulting in sweating [26-28]. Cutaneous vasodilation increases skin blood flow and heat dissipation to the environment. Sweating promotes evaporative heat loss. Both mechanisms reduce core body temperature.

Declining oestrogen production is considered a key trigger of VMS during the menopausal transition. This connection is supported by the effectiveness of oestrogen therapy in alleviating VMS [29]. Furthermore, VMS frequently occur following abrupt oestrogen withdrawal, such as after bilateral oophorectomy or GnRH analogue therapy [26]. However, not all women experience VMS during the menopausal transition, and circulating oestrogen concentrations do not differ between symptomatic and asymptomatic women [30]. These observations suggest that reduced oestrogen exposure alone is insufficient to explain the pathophysiology of VMS and that individual susceptibility and additional neuroendocrine mechanisms are involved.

KNDy neurons and neurokinin signalling

Declining oestrogen concentrations are thought to impair thermoregulatory stability through effects on KNDy neurons. Beyond their established role in mediating oestrogen feedback within the hypothalamic-pituitary-gonadal axis [8], KNDy neurons have also been implicated in thermoregulation and the pathophysiology of VMS. In particular, neurokinin B signalling appears to be important for thermoregulatory control and exerts its effects primarily through the neurokinin 3 receptor (NK3R).

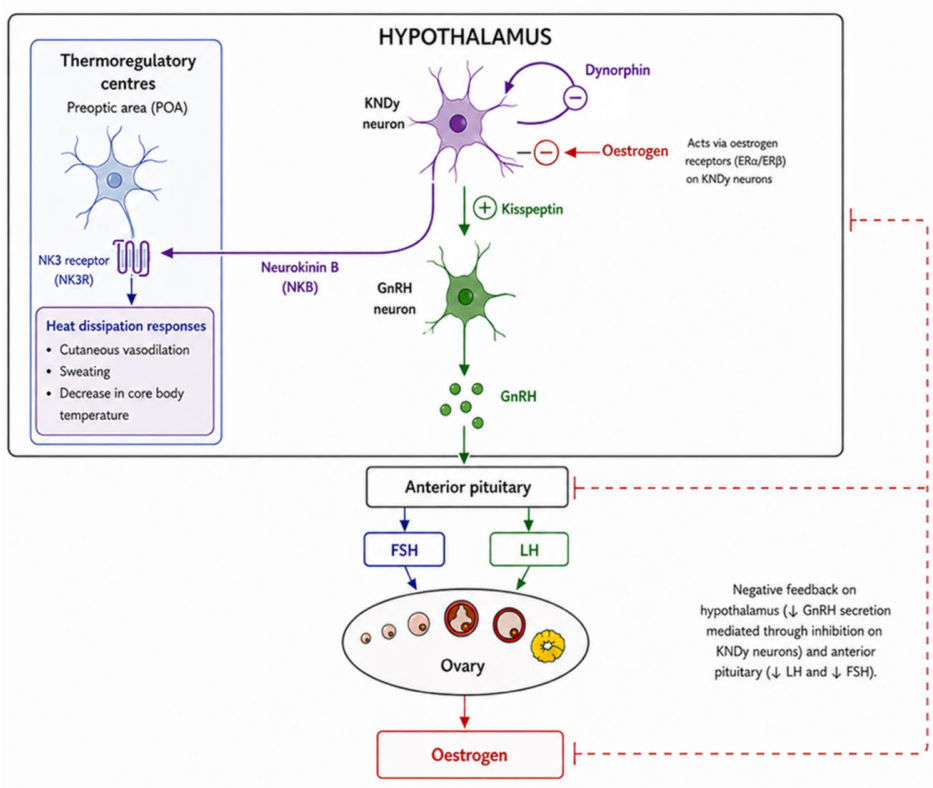

Figure 3. Hypothalamic mechanisms of vasomotor symptoms

Proposed mechanisms linking declining oestrogen concentrations to vasomotor symptoms. Reduced oestrogen-mediated negative feedback increases KNDy neuron activity, resulting in enhanced neurokinin B signalling to thermoregulatory centres and altered GnRH secretion.

During menopausal transition, inhibin and oestrogen and inhibition of KNDy neurons decline. Postmortem studies have demonstrated hypertrophy of KNDy neurons and upregulation of kisspeptin and neurokinin B gene expression and down-regulation of dynorphin in post- compared with premenopausal women, in both natural and surgical menopause [31-33]. Similar changes have been observed in ovariectomized animal models and were reversible with oestrogen treatment [34]. Furthermore, activation of KNDy neurons in mice induced vasodilation and reduced core body temperature, resembling physiological features of a hot flush [35]. This response was enhanced following ovariectomy, whereas pharmacological

blockade of neurokinin B receptors abolished the response [35]. In addition, administration of a selective NK3R agonist into the hypothalamic preoptic area (POA) in rats has been shown to induce increased cutaneous vasomotion and a reduced core body temperature [36]. In healthy premenopausal women, intravenous NKB administration induced hot flushes but did not significantly alter circulating LH or FSH concentrations compared with placebo [37].

Additional evidence implicating the importance of neurokinin signalling comes from genetic studies. Genome-wide association studies have identified variants at the tachykinin receptor 3 (TACR3) locus associated with VMS [38-42]. TACR3 encodes NK3R. These findings provide evidence of inherited susceptibility to VMS and independently implicate the neurokinin pathway. However, genome-wide association studies identify statistical associations and cannot alone establish that the identified variants are functional or causally involved. In the largest genome-wide association study meta-analysis on VMS to date, the leading locus was in TACR3. Collectively, common variants across the genome were estimated explain approximately 8% of the variance in the underlying susceptibility to VMS [42]. This indicates that common genetic variation accounts for a modest proportion of the variation in individual susceptibility to VMS.

No twin study has, to my knowledge, been performed. By comparing concordance between monozygotic and dizygotic twins, such study could estimate the relative contributions of genetic, shared environment, and individual-specific environmental factors. Twin studies would therefore complement genome-wide association studies by providing a broader estimate of genetic and environmental contributions, although they would not identify specific variants or establish biological mechanisms.

These mechanistic findings led to the development of NK3R antagonists for treatment of VMS, which are now licensed in Sweden and used in clinical practice. NK3R antagonists have shown clinically meaningful efficacy in phase 3 trials, reducing moderate-to-severe VMS frequency by approximately 61-64% from baseline to 12 weeks, compared with 35-45% with placebo, corresponding to an additional reduction of approximately 2.5 VMS episodes per day [43-47].

NK3R antagonism has also been shown to modulate gonadotropin secretion. Experimental and clinical studies demonstrated reduced LH secretion and suppression of LH pulsatility following administration of NK3R antagonists, whereas effects on FSH appear less pronounced and less consistent [48-50].

KNDy neurons and endogenous opioids

Dynorphin is an endogenous opioid co-expressed with kisspeptin and neurokinin B in KNDy neurons. Acting through κ-opioid receptors, dynorphin is thought to inhibit KNDy neuronal activity and thereby modulate both GnRH secretion and thermoregulatory signalling [51]. Postmortem studies have demonstrated reduced dynorphin gene expression in the hypothalamus of postmenopausal women [52], suggesting that loss of dynorphin-mediated inhibition during menopause may contribute to increased neurokinin B signalling associated with VMS. Consistent with this hypothesis, a pilot study demonstrated reduced hot flush frequency following treatment with a κ-opioid receptor agonist in postmenopausal women [53].

β-Endorphin is another endogenous opioid that may contribute to hypothalamic control of the reproductive axis. In the human diencephalon, β-endorphin-immunoreactive and GnRH-immunoreactive neural elements form close contacts, mainly in the medial POA and the infundibulum-median eminence [54]. These findings suggest possible regulation of GnRH release, although function of these contacts remain unconfirmed. Endogenous opioid signalling has also been implicated in hypothalamic amenorrhea, which may accompany high exercise loads and low energy availability. In hypogonadotropic women, the opioid antagonist naltrexone 50 mg/day orally for six months increased LH concentrations and pulse amplitude. Menstrual bleeding resumed in most treated women [55]. These effects support opioid inhibition of the gonadotrophin axis but are not specific to β-endorphin. Cerebrospinal fluid β-endorphin concentrations were also lower in postmenopausal than normally cycling women. The concentrations correlated positively with plasma oestradiol across all participants and inversely with

gonadotrophins among cycling women [56]. However, the relevance of β-endorphin to VMS remains uncertain.

Menopausal hormone therapy for vasomotor symptoms

Menopausal hormone therapy (MHT) uses systemic oestradiol to compensate for declining ovarian oestrogen production. It is the most effective treatment currently available for VMS. Women with an intact uterus also require a progestogen to protect the endometrium from proliferation, atypia and potentially endometrial cancer. Oestradiol is administered orally or transdermally and progestogen is administered orally or via an intrauterine device. MHT should be tailored to symptoms, medical history, and individual preferences. In a meta-analysis of randomised placebo-controlled trials, MHT reduced hot flush frequency by 75% relative to placebo. Symptom severity was also substantially reduced [29].

The effect of MHT on quality of life appears to be strongly related to the presence of VMS. In the Heart and Oestrogen/progestin Replacement Study, hormone therapy improved depressive symptoms and quality of life among women reporting VMS, whereas no corresponding benefit was observed among women without VMS [57]. Women without VMS generally reported better quality-of-life measures than women with VMS irrespective of treatment allocation [57]. Similarly, a randomised placebo-controlled trial of postmenopausal women without current or previous VMS, transdermal MHT did not improve psychological well-being compared with placebo, suggesting limited symptomatic benefit in women without VMS [58]. Randomised placebo-controlled studies have otherwise demonstrated concomitant reductions in VMS frequency and improvements in quality of life during MHT, while effects on anxiety, cognitive symptoms, and affective symptoms have been less consistent [59]. Together, these findings suggest that the primary symptomatic benefit of MHT is mediated through alleviation of VMS rather than through broader effects on psychological well-being.

Despite its efficacy, MHT is associated with important risks that must be considered. Large meta-analyses have demonstrated that systemic MHT increases the risk of breast cancer, and that this elevated risk may persist for

more than ten years after cessation of therapy. The risk increases with longer treatment duration, is greater with combined oestrogen-progestagen therapy than with oestrogen-only regimens, and appears higher with continuous progestogen exposure than with intermittent administration [60].

For women of average weight in developed countries who do not use MHT, about 63/1000 will develop breast cancer between the ages 50 and 69. A 5-year regimen of MHT, starting at age 50, results in the following additional cases per 1000 users over the same period: about 20 additional cases (1/50) for oestrogen plus daily progestogen; 14 additional cases (1/70) for oestrogen plus intermittent progestogen; and 5 additional cases (1/200) for oestrogen-only preparations [60]. Furthermore, orally administered MHT is associated with an increased risk for venous thromboembolism, and the risk is higher closer to initiation of therapy [61-63].

Cardiovascular effects of MHT appear to depend on age and timing of treatment initiation. In women initiating MHT within ten years after menopause, the therapy reduces the risk of coronary heart disease and overall mortality compared with placebo or no treatment [61]. In contrast, initiation of MHT more than ten years after menopause has been associated with an increased risk of total stroke (RR 1.21, 95% CI 1.06 - 1.38), an excess risk driven by ischemic stroke [61] .

Some women report side-effects from MHT such as nausea, breast tenderness or irregular bleedings which usually resolve after some time but may cause women to abandon therapy [64].

Non-hormonal treatment strategies are needed for women with VMS who have contraindications to systemic MHT. These include current or previous breast cancer, unexplained vaginal bleeding, and active deep vein thrombosis or pulmonary embolism. Other contraindications include current or previous arterial cardiovascular disease, such as myocardial infarction or stroke, and severe active liver or gallbladder disease. Non-hormonal options are also important for women who experience unacceptable adverse effects or prefer to avoid hormone therapy [65].

Cardiovascular function and the microcirculation

Cardiovascular disease, menopause, and menopausal hormone therapy

CVD is the leading cause of death among women worldwide and the risk of CVD increases after menopause [66]. This increase is believed to reflect not only chronological ageing but also declining endogenous oestrogen concentrations. Earlier menopause has consistently been associated with an increased risk of CVD and CVD mortality, suggesting that longer exposure to endogenous oestrogens may confer cardiovascular protection [67-69]. Similar associations have also been observed following surgical menopause [70]. Furthermore, a meta-analysis demonstrated that early menopause was associated with a modestly increased risk of heart failure [71].

Whether the increased CVD risk associated with menopause can be reversed by exogenous oestrogen administration in the form of MHT remains unclear. For women with early or premature menopause, there are to my knowledge no randomised controlled trial (RCT) with long-term data on CVD outcomes. Nevertheless, MHT is believed to mitigate the cardiovascular effects of premature oestrogen deficiency, largely based on observational data showing an increased mortality in ovariectomised women who did not receive oestrogen replacement, compared with those who did [72]. Regarding MHT and CVD risk in women undergoing menopause at normal age, a Cochrane review was recently published including 24 RCTs and more than 45,000 peri- and postmenopausal women. This meta-analysis concluded that MHT makes little to no difference to the risk of coronary events [73]. However, most women included were older than 60 years, which may limit generalisability of these findings to women in the early menopausal transition. This observation has contributed to the timing hypothesis, which proposes that cardiovascular effects of MHT depend on the timing of treatment initiation relative to menopause, with beneficial effects on atherosclerosis progression observed when treatment is initiated close to menopause but not when initiated 10 or more years later [74]. A 17-year follow-up of the randomised controlled Women's Health Initiative study showed similar results with low CVD risks in the younger age group [75].

Vasomotor symptoms and cardiovascular disease risk

In recent years, VMS *per se* have been suggested to be a risk factor for CVD. Several studies have demonstrated associations between VMS and adverse cardiovascular risk profiles [76], endothelial dysfunction [77], and subclinical atherosclerosis [77-79]. Associations between VMS and clinical CVD outcomes have also been reported, with frequent, persistent, severe, and early-onset VMS being associated with increased risk of subsequent cardiovascular events [80, 81]. However, the underlying mechanisms and clinical significance of these associations remain incompletely understood, and findings across studies have not been entirely consistent [82].

Coronary microcirculatory dysfunction

Historically, research and clinical assessment of ischemic heart disease have focused predominantly on obstructive disease in the epicardial coronary arteries. However, growing evidence indicates that abnormalities of the coronary microcirculation may also contribute to myocardial ischemia, even in the absence of obstructive coronary artery disease. The coronary microcirculation consists of pre-arterioles, intramyocardial arterioles, and capillaries, which together account for most coronary vascular resistance [83, 84]. Its primary function is to continuously adjust myocardial blood flow according to metabolic demand through vasodilatory and vasoconstrictive mechanisms [83, 85, 86].

Coronary microcirculatory dysfunction occurs when these regulatory mechanisms are impaired, resulting in inadequate myocardial perfusion relative to metabolic demand. Both structural and functional abnormalities may contribute, including reduced capillary density, arteriolar remodelling with luminal narrowing, extravascular compression, impaired vasodilation, and enhanced vasoconstriction [83]. Coronary microcirculatory dysfunction is associated with traditional cardiovascular risk factors such as ageing, hypertension, diabetes mellitus, and dyslipidaemia [87]. However, findings from the Women's Ischemia Syndrome Evaluation cohort demonstrated that conventional cardiovascular risk factors explained less than 20% of the variability in coronary vasoreactivity, suggesting that additional mechanisms contribute substantially [87, 88].

Clinically, coronary microcirculatory dysfunction may present with angina and objective signs of myocardial ischemia despite the absence of obstructive epicardial coronary artery disease [89]. Increasing evidence further indicates that coronary microcirculatory dysfunction contributes to the pathophysiology and prognosis of both ischemic heart disease and heart failure with preserved ejection fraction [90-94]. Impaired coronary microvascular function may precede overt clinical deterioration by several years, suggesting that it represents an early stage of CVD progression [94]. Furthermore, patients with impaired coronary microvascular function despite preserved epicardial coronary artery function have been shown to have substantially higher rates of major adverse cardiovascular events than patients with epicardial disease but preserved microvascular function [95].

Assessment of microvascular function

Since the coronary microcirculation cannot be directly visualised *in vivo* because of its small vessel size, its function must instead be assessed indirectly using invasive or non-invasive physiological methods. Invasive coronary function testing performed during cardiac catheterisation remains an important method for assessment of coronary microvascular function [83], although its use is generally restricted to patients undergoing clinically indicated coronary angiography. Non-invasive assessment can be performed using modalities such as positron emission tomography, cardiac magnetic resonance imaging, and transthoracic doppler echocardiography [96-98]. However, these methods are often costly, technically demanding, or have limited availability.

Increasing attention has therefore been directed towards peripheral microvascular beds as surrogate markers of systemic microvascular function. Impaired peripheral microvascular function has been associated with adverse cardiovascular outcomes [99], and coronary microvascular dysfunction has been suggested to represent a systemic rather than solely coronary disorder. This concept is supported by demonstrations of microvascular dysfunction in the cutaneous circulation among patients with cardiac ischemia and no obstructive coronary artery disease [100] and early-onset coronary artery

disease [101]. Furthermore, significant correlations between coronary and cutaneous microcirculatory function have been demonstrated in healthy populations [102].

One method for assessing cutaneous microvascular reactivity is the Enhanced Perfusion and Oxygen Saturation (EPOS) system. EPOS combines diffuse reflectance spectroscopy (DRS) with laser Doppler flowmetry (LDF). It thereby provides simultaneous non-invasive measurements of microvascular oxygen saturation and perfusion [103, 104]. Post-ischaemic peak skin oxygen saturation (OxyP) represents the highest oxygen saturation reached after release of a five-minute arterial occlusion [105]. Higher OxyP is interpreted as greater post-occlusive microvascular reactivity. In the population-based SCAPIS cohort, lower OxyP was associated with a broad range of cardiovascular risk factors. These included smoking, hypertension, diabetes, adiposity, self-reported dyslipidaemia, impaired glycaemic control, and low-grade inflammation [105].

Microcirculation and menopause, menopausal hormone therapy, and vasomotor symptoms

Coronary microcirculatory dysfunction occurs in both men and women, although it appears to be more prevalent among women [106]. Previous studies have also suggested associations between menopausal status, hormonal exposure and microcirculatory function. Postmenopausal women with longer time since menopause demonstrated impaired microvascular reactivity, whereas previous exposure to endogenous or exogenous oestrogens was associated with more preserved microcirculatory function [107]. In addition, acute oestradiol administration has been shown to increase microvascular blood cell velocity and vasodilatory responses in postmenopausal women, with responses being influenced by time since menopause and vasomotor symptoms [108].

Together, these findings suggest that menopausal hormonal changes may influence microcirculatory function, although data remain limited. The associations between cutaneous microvascular function and menopausal status, VMS, and MHT use remain incompletely understood.

Ageing and telomeres

Cellular ageing and telomere biology

Cellular ageing is a fundamental biological process characterised by the progressive accumulation of molecular and cellular damage, ultimately contributing to functional decline and an increased risk of chronic disease. Telomere shortening is one of the most extensively studied hallmarks of ageing [109].

Telomeres are repetitive nucleotide deoxyribonucleic acid (DNA) sequences (TTAGGG) located at the ends of chromosomes, where they preserve chromosomal integrity and protect chromosome ends from being recognised as DNA damage [110-113]. DNA polymerase cannot fully replicate chromosome ends during lagging strand synthesis. This end-replication problem leads to progressive telomere shortening (telomere attrition) with successive cell divisions [110]. Critically short telomeres may trigger cellular senescence, apoptosis, or genomic instability, which limit cellular proliferative capacity and contributing to biological ageing [110, 113, 114]. Experimental studies have demonstrated that maintenance of telomere length through expression of telomerase, a ribonucleoprotein enzyme complex that adds telomeric DNA repeats to chromosome ends, can prevent replicative senescence, supporting a causal role of telomere shortening in cellular ageing [114]. However, because telomerase activity is low or absent in most somatic tissues, telomeres continue to shorten throughout life [115]. Consistent with this mechanism, telomere length decreases with advancing age *in vivo* [116]. Telomere attrition is further accelerated by oxidative stress, inflammation, and other forms of cellular stress that promote DNA damage and cellular turnover [117, 118].

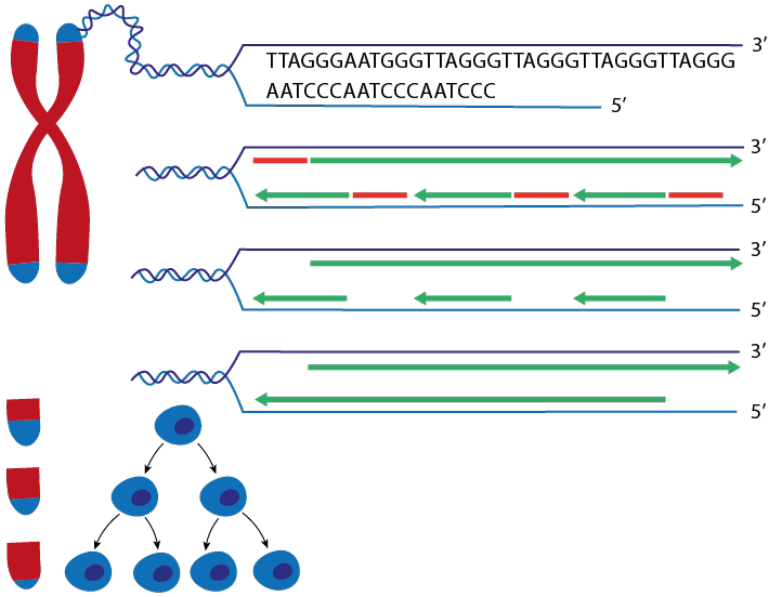

Figure 4. The end-replication problem and progressive telomere shortening

During DNA replication, the lagging strand is synthesised discontinuously from RNA primers. After removal of the terminal primer, DNA polymerase cannot fill the remaining gap, leaving the newly synthesised strand shorter than its template. In cells without sufficient telomerase activity, this process results in progressive telomere shortening with successive cell divisions. Blue chromosome ends represent telomeres, red segments represent RNA primers, and green segments represent newly synthesised DNA.

Assessment of telomere length

Telomere length varies across tissues and does not uniformly reflect biological ageing [119]. Whereas telomeres undergo little shortening in postmitotic tissues such as skeletal muscle, progressive shortening occurs in proliferative tissues such as the liver [119]. In the liver, telomere shortening has been associated with chronic liver disease such as cirrhosis, illustrating that telomere dynamics may reflect tissue-specific biological processes [120]. Although telomere length dynamics within individual organs may reflect local processes, leukocyte telomere length (LTL) has consistently been associated with general age-related disease [121-125] and mortality [126] in

population studies and has therefore become the most widely used measure of telomere length in research on human ageing. LTL is derived from circulating leukocytes, which are continuously replenished from the haematopoietic stem cell compartment throughout life [119]. Although substantial inter-individual variation exists and LTL is influenced by both genetic and environmental factors, twin studies have demonstrated that associations between shorter LTL and mortality persist within twin pairs, suggesting that this relationship is not fully explained by shared genetic or early-life environmental factors [127, 128].

LTL can be assessed using several laboratory methods, including Southern blot, fluorescence in situ hybridisation, and quantitative polymerase chain reaction (qPCR) [129]. Among these, qPCR has become the most widely used method because it requires only small amounts of DNA and allows rapid processing of large numbers of samples [129].

Telomeres, oestrogen, and menopause

Adult women generally have longer telomeres than men of similar age, a difference that has been hypothesised to be mediated by oestrogen [130]. Oestrogen may contribute to the preservation of telomere length through activation of telomerase [131, 132] and/or through antioxidative effects [133, 134]. Supporting a potential role of oestrogen in telomere maintenance, long-term MHT use was associated with longer telomere length in postmenopausal women [135]. A large recent study reported an association between earlier menopause and shorter LTL [136]. However, findings across the literature remain inconsistent, and a recent review concluded that the relationship between menopause and telomere length is not yet fully understood [137]. Nevertheless, postmenopausal women may provide a useful model for investigating determinants of telomere length, as the relatively stable low oestrogen concentrations observed after menopause minimise any potential influence of cyclical hormonal variation or change during menopausal transition.

Physical activity

Table 1. Definitions of terms related to physical activity, exercise, and sedentary behaviour

Term	Definition
Physical activity	Any bodily movement produced by skeletal muscles that requires energy expenditure.
Physical exercise	A planned, structured, and repetitive form of physical activity undertaken to improve or maintain one or more components of physical fitness.
Aerobic physical activity	Activity involving rhythmic movement of large muscle groups over a sustained period, thereby improving cardiorespiratory fitness. Examples including walking, running, cycling, and swimming.
Resistance training	A form of physical exercise in which muscles contract against external resistance, such as free weights, machines, resistance bands or body weight, to improve muscular strength, power, endurance, or other aspects of muscle function.
Physical inactivity	A level of physical activity that is insufficient to meet current physical activity recommendations.
Metabolic equivalent of task (MET)	A measure of the energy expenditure associated with physical activity. One MET represents resting energy expenditure and is conventionally approximated as an oxygen consumption of 3.5mL/kg/min.
Sedentary behaviour	Any waking behaviour characterised by an energy expenditure of ≤1.5 METs while sitting, reclining, or lying.
Light-intensity physical activity	Physical activity performed at 1.5 to <3 METs. On a relative scale about 2-4 on a rating scale of perceived exertion of 1-10. E.g. slow walking or other activities that do not result in substantial increase in heart rate or breathing rate.
Moderate-intensity physical activity	Physical activity performed at 3 to <6 METs. On a relative scale about 5-6 on a rating scale of perceived exertion of 1-10.
Vigorous physical activity	Physical activity performed at ≥6 METs. On a relative scale >6 on a rating scale of perceived exertion of 1-10.
Moderate-to-vigorous physical activity (MVPA)	Physical activity performed at ≥3 METs, encompassing both moderate- and vigorous-intensity acitivty. On a relative scale ≥5 on a rating scale of perceived exertion of 1-10.

Definitions of physical activity, physical activity intensity, and sedentary behaviour were adapted from the World Health Organization guidelines on physical activity and sedentary behaviour [138]. The

definition of exercise was adapted from Caspersen et al. [139], and the definition of resistance training from the American College of Sports Medicine position stand [140].

Physical activity encompasses movement performed in daily life as well as structured exercise. As these related terms are not interchangeable, the definitions used throughout this thesis are summarised in table 1.

Physical activity and disease prevention

Physical inactivity is a major modifiable risk factor for premature mortality and non-communicable disease. Large epidemiological studies have consistently demonstrated that higher levels of physical activity are associated with lower all-cause mortality, with similar associations observed across populations and age groups [141, 142]. More recent studies using objectively measured physical activity have strengthened these findings and demonstrated dose-dependent associations with mortality risk [141]. Importantly, adopting a physically active lifestyle later in adulthood may still substantially reduce mortality risk compared with remaining inactive [143].

Although RCTs in primary prevention settings have generally failed to demonstrate reductions in mortality or cardiovascular events, exercise interventions consistently improve several established cardiovascular risk factors, including blood pressure, lipid profile, glucose metabolism, body composition, and cardiorespiratory fitness [144-146]. In contrast, exercise-based cardiac rehabilitation in patients with established coronary heart disease has been shown to reduce the risk of recurrent myocardial infarction, all-cause hospitalisation and mortality [147]. Together, evidence from observational studies, intervention studies, and mendelian randomisation studies that use genetic variants as proxies for physical activity to strengthen causal inference, supports a beneficial role of physical activity in disease prevention and healthy ageing [144-148].

The beneficial effects of physical activity are partly mediated through improvements in established cardiovascular risk factors, including blood pressure, lipid profile, insulin sensitivity, and body composition [149]. However, improvements in traditional risk factors appear to explain only part

of the reduction in cardiovascular risk associated with exercise [149, 150]. This suggests that physical activity may also exert effects on the vasculature and other biological systems that contribute to its protective effects [149, 150].

Physical activity and microvascular function

A systematic review and meta-analysis reported a moderate beneficial effect of exercise on cutaneous microvascular reactivity, although the analysis was based on a limited number of relatively small studies [151]. Consistent with these findings, aerobic exercise has been shown to improve endothelium-dependent cutaneous vasodilation and increase circulating nitric oxide metabolites in previously sedentary adults [152].

Exercise-induced improvements in cutaneous microvascular function have also been reported in postmenopausal women, with microvascular responses approaching those observed in younger women after prolonged (48 weeks) endurance training [153]. However, evidence regarding the relationship between habitual physical activity and microvascular function in population-based cohorts of midlife women remains limited.

Physical activity and telomeres

Observational studies have generally reported longer LTL among physically active individuals than their less active counterparts, and positive associations have been observed after adjustment for several important confounders [154]. Consistent with these findings, a large cross-sectional study of more than 5000 adults reported significantly longer LTL among individuals with high levels of physical activity compared with sedentary participants, corresponding to an estimated biological ageing advantage of approximately nine years. The association remained significant after adjustment for demographic and lifestyle-related covariates [155]. Similar findings have been reported in postmenopausal women [156]. In a small cross-sectional study of healthy postmenopausal women, those who regularly participated in combined aerobic and resistance exercise for more than 12 months exhibited longer LTL than sedentary women, and habitual exercise was independently associated with telomere length after adjustment for potential confounders

[156]. However, evidence regarding the association between physical activity and telomere length remains inconsistent. A systematic review and meta-analysis found considerable heterogeneity between studies and no significant overall association between physical activity and LTL, concluding that the available evidence was insufficient to establish a clear relationship [157].

A randomised trial showed that endurance exercise and high-intensity interval training may increase both telomerase activity and LTL [158]. However, intervention studies remain scarce. Notably, resistance training has been investigated in only one prior randomised study, which reported no effect on LTL [158]. It would therefore be of interest to assess LTL prospectively in a RCT involving postmenopausal women with stable and low oestrogen levels.

Physical activity as a treatment for vasomotor symptoms

Physical exercise was first suggested to be associated with a lower prevalence of VMS in 1990, and subsequent observational studies reported similar findings [159, 160]. Although evidence from intervention studies remains limited, several exercise interventions have reported reductions in VMS frequency following exercise interventions [161-164]. A Cochrane review published in 2014 concluded that available evidence was insufficient to determine whether exercise is an effective treatment for VMS [165]. More recently, a Swedish RCT demonstrated that 15 weeks of supervised resistance training reduced VMS frequency by approximately 50% in postmenopausal women [166].

The mechanisms underlying a potential effect of exercise on VMS remain incompletely understood. Experimental studies suggest that exercise may influence hypothalamic pathways involved in thermoregulation including KNDy neuron signalling, a pathway implicated in both thermoregulation and regulation of GnRH. In one animal model, exercise was associated with reduced GnRH messenger ribonucleic acid (mRNA) expression together with alterations in neurokinin B and dynorphin expression within the arcuate nucleus [167]. In another study, the effects of aerobic exercise and oestrogen treatment were compared in ovariectomised rats, a model of oestrogen deficiency [168]. Exercise reduced tail skin temperature, whereas untreated

animals exhibited the highest temperatures. Notably, only the exercise group showed reduced expression of kisspeptin, neurokinin B, and prodynorphin in the arcuate nucleus. The authors proposed that exercise may attenuate thermoregulatory deficiency through modulation of KNDy neuron signalling [168].

Collectively, the above-mentioned findings suggest that exercise may influence KNDy neuron activity, a pathway involved in both thermoregulation and regulation of the gonadotropin-releasing hormone pulse generator. Modulation of KNDy neuron signalling may therefore represent a potential mechanism through which exercise alleviates VMS. Given the role of KNDy neurons in the regulation of gonadotropin secretion, such effects may also be reflected by changes in circulating LH and FSH concentrations. However, the effects of exercise on gonadotropin concentrations in postmenopausal women remain largely unexplored but should be investigated to clarify the mechanisms behind reduced VMS in postmenopausal women participating in physical exercise.

Maintenance of physical activity

Despite the well-established health benefits of physical activity, insufficient physical activity remains highly prevalent worldwide. In 2022, 31.3% of adults were estimated to have insufficient physical activity levels globally, with a higher prevalence among women than men. Furthermore, the prevalence of insufficient physical activity has increased over time, particularly among older adults [169].

Although many interventions successfully increase physical activity in the short term, maintenance of physical activity remains a major challenge. Physical activity maintenance is recognised as a distinct process from behaviour initiation and may be influenced by partly different determinants [170]. A recent systematic review and meta-analysis of behavioural interventions with at least 24 months of follow-up found small but clinically meaningful increases in objectively measured physical activity that persisted up to 48 months [171]. However, the effect sizes were small, highlighting the

challenges of achieving substantial long-term increases in physical activity [171].

Individual differences in physical activity behaviour seem to be influenced by both genetic and environmental factors. In a large twin study using objective measures of physical activity, heritability accounted for 47% of the variance in physical activity energy expenditure, whereas most of the remaining variance was explained by unique environmental factors and random error. Shared environmental factors contributed little [172]. Genetic influences may partly act through factors that promote engagement in physical activity, as both twin studies and genome-wide association studies suggest that exercise ability, exercise enjoyment, and motives for physical activity contribute to the heritability of physical activity behaviour [173, 174]. Nevertheless, environmental influences account for most of the variation in motives for physical activity [173], indicating that physical activity behaviour remains modifiable and may be responsive to appropriately designed interventions.

Despite increasing interest in long-term behaviour change, data on maintenance of physical activity following supervised exercise interventions remain limited. Long-term follow-up studies are therefore needed to determine the extent to which increases in physical activity achieved during an intervention are sustained after formal supervision has ended.

THESIS RATIONALE

Despite extensive evidence supporting the health benefits of physical activity, important questions remain regarding its role during the menopausal transition. While exercise has been proposed as a potential strategy for improving health and well-being in midlife women, evidence regarding long-term maintenance, underlying mechanisms on the effects on VMS, and broader physiological effects remains incomplete.

The studies included in this thesis were designed to address these knowledge gaps from complementary perspectives. Using both intervention and population-based data, the thesis examines whether physical activity can be sustained long term; potential neuroendocrine responses to resistance training and their relationship with changes in VMS; differences in microvascular function across groups defined by menopausal characteristics, physical activity, and sedentary behaviour; and whether resistance training affects telomere length. Together, these studies aim to contribute to a broader understanding of the role of physical activity in climacteric women.

HYPOTHESIS

General hypothesis

Physical activity contributes to favourable health-related adaptations in climacteric women.

Specific hypotheses

- An increase in physical activity following a resistance training intervention can be sustained for two years in postmenopausal women with VMS.
- Resistance training reduces circulating gonadotrophin concentrations in postmenopausal women with VMS.
- Among women who have never used MHT, premenopausal women have higher OxyP than postmenopausal women.
- Postmenopausal women currently using MHT have higher OxyP than postmenopausal women who have never used MHT.
- OxyP differs across VMS frequency groups in postmenopausal women. The direction of the differences is not prespecified.
- Midlife women with higher levels of physical activity and lower levels of sedentary behaviour have higher OxyP.
- Resistance training increases LTL or attenuates LTL shortening over 15 weeks compared with an untreated control in postmenopausal women with VMS and low physical activity at baseline.
- Higher physical activity during follow-up is associated with longer LTL at two years.
- Women randomised to resistance training have longer LTL at two years than those randomised to the control group.

AIMS

General aim

To investigate the role of physical activity in climacteric women, with a particular focus on whether increased physical activity can be sustained long term, circulating gonadotrophin concentrations, cutaneous microvascular reactivity, and LTL as a marker of cellular ageing.

Specific aims

- To examine whether an increase in physical activity can be sustained for two years following a resistance training intervention in postmenopausal women with VMS.
- To determine whether a structured 15-week resistance training intervention decreases circulating gonadotrophin concentrations in postmenopausal women with VMS.
- To compare OxyP across groups defined by menopausal status, MHT use, VMS frequency, physical activity, and sedentary behaviour in midlife women.
- To investigate the effect of 15 weeks of resistance training on LTL in postmenopausal women with VMS and low physical activity at baseline, compare LTL change from baseline to two years between the original randomisation groups, and examine whether physical activity during follow-up is related to LTL change from 15 weeks to two years.

MATERIAL AND METHODS

Study overview

This thesis is based on four studies investigating physical activity, VMS, microvascular function, gonadotropin concentrations, and telomeres in midlife and postmenopausal women. An overview of the included studies is presented in Table 2.

Table 2. Overview of the four studies that form the basis of this thesis

	Sustained physical activity	Gonadotrophin concentrations	Microvascular function	Telomere length
Study design	2-year RCT follow-up	Open RCT	Cross-sectional	Open RCT with two-year follow-up
Population	65 postmenopausal women with VMS and low PA at baseline	65 postmenopausal women with VMS and low PA at baseline	1072 women aged 50-64 years from SCAPIS	65 postmenopausal women with VMS and low PA at baseline
Intervention/ exposure	15-week RT; subsequent support to both groups	15-week RT	Menopause, MHT, VMS, MVPA, sedentary time	15-week RT; mean MVPA during follow-up
Comparison	Changes over time within and between original groups	15-week change: RT (ITT and PP) versus control	Differences across predefined groups	LTL change by allocation, and follow-up MVPA.
Main outcome	PA over two years	Plasma LH and FSH	OxyP	Change in LTL
Assessment methods	IPAQ	Immunoassays	Questionnaire, accelerometry, and EPOS	qPCR and IPAQ

Abbreviations: EPOS, Enhanced Perfusion and Oxygen Saturation; FSH, follicle-stimulating hormone; IPAQ, International Physical Activity Questionnaire; ITT, intention-to-treat; LH, luteinising hormone; LTL, leucocyte telomere length; MHT, menopausal hormone therapy; MVPA, moderate-to-vigorous physical activity; OxyP, peak oxygen saturation after release of arterial occlusion; PA, physical activity; PP, per-protocol; qPCR, quantitative polymerase chain reaction; RCT, randomised controlled trial; RT, resistance training; SCAPIS, Swedish CArdioPulmonary bioImage Study; VMS, vasomotor symptoms.

Study designs and populations

The STYRKA trial

The STYRKA trial was an open, parallel-group RCT evaluating a 15-week resistance training intervention in postmenopausal women with VMS. Participants were recruited through newspaper advertisements and posters displayed at Linköping University Hospital. Eligible women were aged 45 years or older, were postmenopausal, and recorded a mean of at least four moderate-to-severe VMS per 24 hours in a daily diary during a two-week screening period. They had low physical activity at baseline, defined as no more than 75 minutes of MVPA or 225 minutes of physical activity of any intensity per week. Further eligibility criteria included no recent treatment for VMS, generally good health, and sufficient physical capacity to participate in resistance training.

Participants were randomised in a 1:1 ratio to a 15-week resistance training intervention or an untreated control condition in which they were instructed to maintain their usual level of physical activity. The sample size calculation indicated that 40 participants were required after allowing for an expected dropout rate of 20%. The recruitment target was subsequently increased to 60 to improve statistical power. Overall, 65 women were randomised between November 2013 and October 2016. After the initial 15-week controlled period, women originally allocated to the control group were offered an introductory resistance training session and four months of gym access. Both original randomisation groups were subsequently followed for two years.

Together, the controlled intervention and subsequent follow-up allowed us to examine sustained physical activity, gonadotrophin responses, and changes in LTL. The LTL analyses compared the original randomisation groups and examined physical activity during follow-up in relation to LTL change.

Resistance training intervention

The intervention comprised 15 weeks of whole-body resistance training, with three sessions per week. Each session included six machine-based exercises—chest press, leg press, seated row, leg curl, latissimus dorsi pull-

down, and leg extension—and two bodyweight exercises, crunches and back raises. The machine-based exercises were performed in two sets, with two minutes of rest between sets. During the first three weeks, participants performed 15–20 repetitions at 15–20 RM to allow gradual adaptation and reduce the risk of injury. Thereafter, the load was increased to 8–12 RM, with 8–12 repetitions per set. The bodyweight exercises were performed in two sets to volitional exhaustion. Loads were individualised based on strength testing conducted by the study physiotherapist at baseline and after three weeks and were progressively adjusted throughout the intervention. Sessions lasted approximately 45 minutes, including a 7–10-minute warm-up and concluding stretching and mobility exercises.

Participants trained independently at a local gym, with one session per week supervised by the physiotherapist. Attendance was monitored through the gym's electronic access system, and participants recorded the exercises, loads, sets, and repetitions completed in individual training diaries.

During the initial 15-week controlled period, women allocated to the control group were instructed to maintain their baseline level of physical activity and not to initiate any new treatment for VMS. After this period, they were offered an individual introduction to resistance training, a personalised training programme, a training diary, and four months of free gym access. Women originally allocated to the intervention group were encouraged to continue training and were offered an additional consultation with the physiotherapist to update their programme. During the subsequent two-year follow-up, both original randomisation groups were encouraged to remain physically active and received information and supportive emails every six months.

Swedish CArdioPulmonary bioImage Study (SCAPIS)

SCAPIS is a nationwide, population-based cohort study designed to investigate cardiovascular disease and chronic obstructive pulmonary disease. Between 2013 and 2018, 30,154 men and women aged 50–64 years underwent baseline examinations at six Swedish university hospitals. Participants were randomly selected from the population register within each recruitment area. Examinations included cardiovascular and pulmonary

imaging, physiological assessments, blood sampling, and questionnaires on health and lifestyle.

To compare OxyP across groups defined by physical activity level, menopausal status, VMS frequency, and current MHT use, we conducted a cross-sectional study. The Linköping site of SCAPIS included 2,526 women aged 50–64 years. Of these, 767 had invalid or missing OxyP measurements. A valid measurement of peak oxygen saturation after release of arterial occlusion (OxyP) was available for the remaining 1,759 women.

We excluded 161 women with former MHT use and 85 with missing MHT data. A further 110 women were excluded because they were perimenopausal. Menopausal status was uncertain because of previous gynaecological surgery in 107 women and hormone treatment in 221 women. These women were excluded, together with three women whose menopausal status was missing. After these exclusions, the study population comprised 1,072 women.

Exposures

Menopausal status, menopausal hormone therapy use, and vasomotor symptoms

Information on menopausal status, MHT use, and VMS was obtained from the Survey on Women's Health, an additional questionnaire administered to female SCAPIS participants in Linköping. The Swedish questionnaire is included as an appendix to this thesis. Premenopausal women reported menstruation during the preceding year without an interruption lasting at least three consecutive months, whereas postmenopausal women reported no menstruation during the preceding year. Perimenopause was defined as an interruption of menstruation for at least three consecutive months during the preceding year.

Current MHT use was defined as current use of systemic MHT. Never users reported no current or previous systemic MHT use. Menopausal status was compared between premenopausal and postmenopausal women who had never used MHT. MHT use was compared between current and never users among postmenopausal women; former users were excluded. Women with

missing MHT data, perimenopausal status, or uncertain menopausal status due to previous gynaecological surgery or hormone treatment were excluded from the relevant comparisons.

VMS frequency was examined among postmenopausal women who had never used MHT. Participants reported the frequency of hot flushes and/or night sweats during the preceding two weeks and were classified as having no current VMS, less than daily VMS, or daily VMS. The less than daily VMS category included symptoms occurring occasionally or a few times per week. The daily category included symptoms occurring once or several times per day.

Accelerometer-measured physical activity and sedentary behaviour

Physical activity and sedentary behaviour were assessed using a triaxial ActiGraph accelerometer (ActiGraph LLC, Pensacola, FL, USA). The accelerometer was worn over the right hip during waking hours for seven consecutive days, except during water-based activities. Recordings were considered valid when wear time was at least 600 minutes per day on at least four days.

Moderate-to-vigorous physical activity (MVPA) was defined as ≥2,690 counts per minute (cpm), and sedentary time as <200 cpm. Prolonged sedentary time was defined as time accumulated in uninterrupted bouts of at least 20 minutes below 200 cpm. MVPA and prolonged sedentary time were each categorised into tertiles.

Outcomes and outcome assessment

Long-term physical activity

Physical activity during the 15-week RCT and subsequent two-year follow-up was assessed using the International Physical Activity Questionnaire (IPAQ) short form [175, 176]. IPAQ was administered at baseline, after 15 weeks, and at the six-month and two-year follow-ups, with follow-up intervals measured from the end of the 15-week intervention. Participants reported the frequency and duration of walking, moderate-intensity physical activity, and vigorous-intensity physical activity performed during the

previous seven days. Physical activity was categorised as low, moderate, or high according to the standard IPAQ scoring protocol [176].

Total and intensity-specific physical activity were expressed as MET-minutes per week. These values were calculated by multiplying the reported weekly duration of each activity category by its assigned MET value; the intensity-specific values were summed to obtain total MET-minutes per week. MVPA was calculated as the combined duration of moderate- and vigorous-intensity physical activity and expressed as minutes per week. The IPAQ item assessing sitting time was excluded because of extensive missing data. When at least one activity category had been completed, blank responses for the remaining activity categories were assigned a value of zero minutes.

For the analysis of physical activity in relation to LTL change, mean MVPA was calculated from the six-month and two-year assessments. If only one assessment was available, that value was used.

Gonadotrophin concentrations

Plasma concentrations of LH and FSH were assessed to investigate potential neuroendocrine effects of resistance training. Fasting venous blood samples were collected at baseline and at the week-15 visit. Blood was collected in ethylenediaminetetraacetic acid (EDTA) Vacutainer tubes (BD AB, Sweden) and centrifuged at 1,500 × *g*. Plasma was aliquoted and stored at −70 °C until analysis. All plasma samples were subsequently thawed and analysed in a single batch. LH and FSH were analysed according to routine procedures at an accredited clinical chemistry laboratory (ISO/IEC 17025) at Linköping University Hospital.

Cutaneous microvascular reactivity

Cutaneous microvascular reactivity was assessed using the PeriFlux 6000 EPOS system (Perimed AB, Järfälla, Sweden), a non-invasive optical system combining laser LDF and DRS. Participants rested in a supine position for at least 15 minutes before the examination. A fibre-optic probe was placed on the volar side of the right forearm, and microvascular oxygen saturation and perfusion were recorded continuously during a 20-minute occlusion–release

protocol consisting of 5 minutes of baseline recording, 5 minutes of brachial arterial occlusion at 250 mmHg, and 10 minutes of post-occlusive reperfusion. Inflation of the cuff interrupted arterial blood flow, whereas its release elicited post-occlusive reactive hyperaemia, reflecting the capacity of the microvasculature to respond to transient ischaemia.

OxyP, defined as the highest oxygen saturation recorded after cuff release, was used as the measure of cutaneous microvascular reactivity. OxyP was compared between groups defined by menopausal status and MHT use and across groups defined by VMS frequency, MVPA, and prolonged sedentary time. The microcirculatory assessment protocol has been described in detail elsewhere [177].

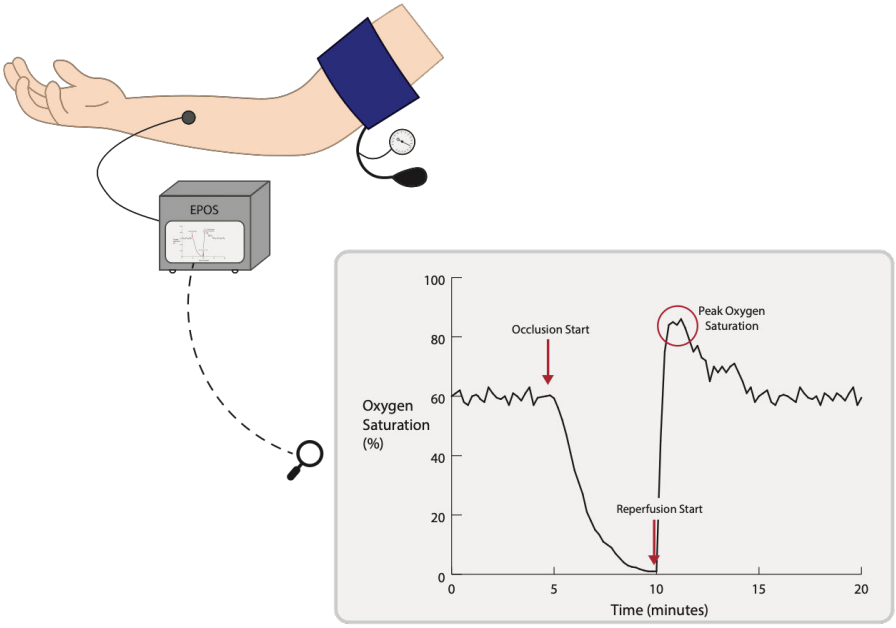

Figure 5. Measurement of post-ischemic peak skin oxygen saturation (OxyP)

A fibre-optic Enhanced Perfusion and Oxygen Saturation (EPOS) probe was placed on the volar forearm, and an occlusion cuff was applied to the upper arm. After a 5-min baseline period, the cuff was inflated to 250 mm Hg for 5 min and then released, followed by 10 min of reperfusion. Skin microvascular oxygen saturation was measured continuously. OxyP was defined as the highest oxygen saturation recorded after cuff release. The oxygen saturation curve is schematic.

To standardise the measurements, participants were instructed to refrain from coffee and large meals for 3 hours, nicotine for 4 hours, and alcohol for 12 hours before the examination. They were also instructed to avoid taking medication before the examination on the study day, except for anticoagulants, contraceptives, and medications for Parkinson's disease, diabetes, epilepsy, chronic pain, or spasticity [177]. Participants subsequently rested in a supine position for at least 15 minutes before the examination.

Telomere length

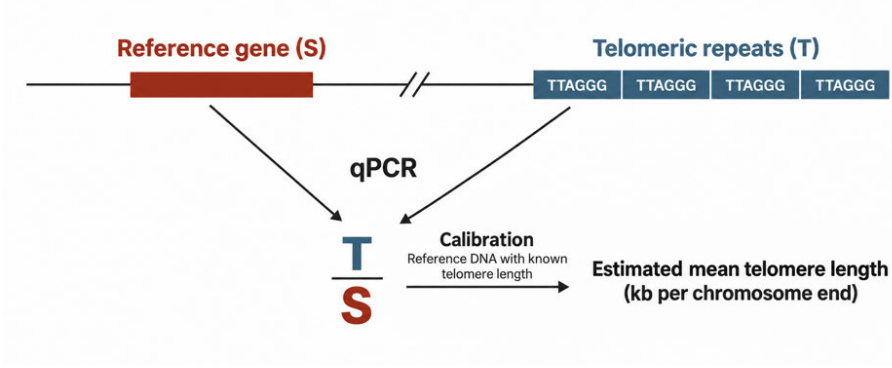

Figure 6. Principle of telomere length measurement using quantitative polymerase chain reaction

Quantitative polymerase chain reaction (qPCR) separately amplifies telomeric repeats and the single-copy reference gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The telomere signal is normalised to the reference-gene signal to account for differences in genomic DNA input. This yields a relative measure of telomere content, expressed as the T/S ratio. The resulting values were calibrated against a reference sample with a known mean telomere length. This calibration provided an estimate of mean leucocyte telomere length, expressed in kilobase pairs per chromosome end. Blue segments represent telomeric TTAGGG repeats, and the red segment represents the single-copy reference gene.

LTL was assessed at baseline, after the 15-week intervention, and at the 2-year follow-up to evaluate changes during the intervention and subsequent follow-up period. The additional blood-sampling visit took place 24–28 months after the 15-week visit. This assessment was introduced after recruitment had begun, and women who had already exceeded this interval

could not be invited. Fasting venous blood samples were collected at each assessment. Genomic DNA was extracted from peripheral blood leucocytes and stored at −30°C until analysis.

LTL was measured using qPCR. Telomeric repeats and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), used as the single-copy reference gene, were amplified separately. Samples from the same participant were analysed within the same batch and in triplicate. An efficiency-adjusted ΔΔCt method was used to quantify the telomere-repeat signal relative to the single-copy reference-gene signal. The resulting values were calibrated against a reference sample with a known mean telomere length to estimate LTL, which was expressed in kilobase pairs per chromosome end.

Covariates

Analyses of cutaneous microvascular reactivity

The covariates included age, body mass index (BMI), smoking status, and diabetes. Information on age, smoking status, and self-reported diabetes was obtained from the SCAPIS questionnaire. Height and weight were measured using standardised SCAPIS procedures, and BMI was calculated as weight in kilograms divided by height in metres squared (kg/m**²**). Diabetes was defined as a fasting plasma glucose concentration of ≥7.0 mmol/L, a glycated haemoglobin (HbA1c) concentration of ≥48 mmol/mol, or a self-reported diagnosis of diabetes. Participants were classified as current, former, or never smokers.

Statistical analyses

Continuous variables are presented as mean (standard deviation [SD]) or median (first quartile–third quartile [Q1–Q3]), as appropriate for their distributions. Categorical variables are presented as numbers (percentages).

Changes in physical activity outcomes over time within each randomised group were analysed using the Friedman test, followed by post hoc Wilcoxon signed-rank tests. Between-group comparisons at each time point were performed using the Mann–Whitney U test.

Absolute changes in gonadotrophin concentrations from baseline to week 15 were compared between the intervention and control groups using the Mann–Whitney U test. Comparisons were performed according to both the intention-to-treat (ITT) and per-protocol (PP) principles. The PP analysis included intervention participants who completed an average of at least two resistance training sessions per week, in accordance with the prespecified compliance criterion.

Unadjusted differences in OxyP between two groups were examined using independent-samples t-tests, whereas comparisons involving three groups used one-way analysis of variance. Adjusted analyses used analysis of covariance. Model 0 was unadjusted; Model 1 was adjusted for age; Model 2 was adjusted for age and BMI; and Model 3 was adjusted for age, BMI, smoking status, and diabetes. Pairwise post hoc comparisons were performed only when the overall test was statistically significant and were adjusted for multiple comparisons using the Bonferroni method.

LTL change was calculated by subtracting the earlier measurement from the later measurement. Linear regression compared changes from baseline to 15 weeks and from baseline to two years between the original randomisation groups. Both comparisons were adjusted for baseline LTL.

For the observational analysis, both groups were pooled. LTL change from 15 weeks to two years was regressed on mean follow-up MVPA, with adjustment for LTL at 15 weeks. MVPA coefficients were expressed per 60 min/week. All three models were additionally adjusted for baseline age in separate analyses. Each model included participants with the required paired LTL measurements and covariate data.

Residual plots and influence diagnostics were examined. Sensitivity analyses excluded influential observations individually. For the baseline-adjusted group comparisons, 5000 bootstrap resamples yielded bias-corrected and accelerated 95% confidence intervals. Further MVPA analyses required both activity assessments or used the six-month assessment alone. Regression estimates were reported with 95% confidence intervals. No multiplicity

adjustment was applied to the LTL analyses, and follow-up and sensitivity analyses were interpreted as exploratory.

Apart from the IPAQ coding described above, missing data were not imputed. Analyses used available observations, with complete pairs required for each LTL interval. All statistical tests were two-sided, and p values <0.05 were considered statistically significant. Analyses were performed using IBM SPSS Statistics versions 24, 26, 28, and 31 (IBM Corp., Armonk, NY, USA).

The sample-size calculation for the STYRKA trial was based on the change in mean moderate-to-severe VMS frequency observed among the first 16 participants. Based on the observed standard deviations and an alpha level of 0.025, 32 participants were required to detect a 50% between-group difference with 80% power. Allowing for an anticipated dropout rate of 20% increased the required sample to 40 participants, with 20 participants per group. A reduction of at least 50% in VMS frequency was considered clinically relevant [178]. The recruitment target was subsequently increased to 60 participants to increase statistical power, particularly for secondary outcomes. All eligible women responding to the final advertisement were included, resulting in a total sample of 65 participants. No separate outcome-specific sample-size calculation was performed for sustained physical activity, gonadotrophin concentrations, or LTL.

Randomisation and blinding

The allocation sequence for the RCT was generated by a statistician independent of the study team using computer-generated block randomisation in blocks of four with a 1:1 allocation ratio (Stata version 13.1; StataCorp LP, College Station, TX, USA). Group allocation was concealed in opaque, sealed, sequentially numbered envelopes, which were stored in a locked location and handled by a research nurse. After eligibility had been confirmed, the next sequentially numbered envelope was retrieved and opened in the presence of the participant and a member of the research team. Participants and the supervising physiotherapist were not blinded. Laboratory personnel measuring LTL were blinded to allocation, whereas the statistical analyses were not blinded.

Ethical approvals and considerations

All research included in this thesis was conducted in accordance with the principles of the Declaration of Helsinki. The STYRKA trial, including the analyses of sustained physical activity, gonadotrophin concentrations, and LTL, was approved by the Regional Ethical Review Board in Linköping, Sweden (Dnr 2013/285-31; two-year follow-up: Dnr 2017/73-32). SCAPIS and the additional data collection and analyses conducted in Linköping were approved by the Regional Ethical Review Boards in Umeå and Linköping and, following the reorganisation of the Swedish ethical review system, by the Swedish Ethical Review Authority (Dnr 2010-228-31M, 2015-246/32, 2018/156-31, 2018/478-31, 2020-03101, and 2021-07061-02). All participants received oral and written information about the relevant study and provided written informed consent before undergoing any study-specific procedures.

The STYRKA RCT compared resistance training with a control condition in postmenopausal women with bothersome VMS. Foreseeable risks of resistance training included musculoskeletal discomfort and exercise-related injuries. To minimise these risks, participants in the intervention group received individual instructions from a physiotherapist, who also supervised one training session per week. Venous blood sampling entailed a risk of transient pain, bruising, or discomfort. Participants were asked not to initiate treatment for VMS during the 15-week intervention period because such treatment could influence the outcomes. Participants allocated to the control group were instructed to maintain their usual physical activity during this period but were subsequently offered an introduction to the resistance training programme and a four-month gym membership. Participation was voluntary, and participants could withdraw at any time without providing a reason and without affecting their subsequent care.

Data for the cross-sectional analyses were derived from SCAPIS, a population-based study in which participants were randomly selected from the general population and invited to participate. The study involved extensive clinical examinations, imaging procedures, and biological

sampling. Because participants were recruited from the general population rather than seeking medical care, the balance between potential risks and the anticipated scientific and societal value required careful consideration. Although most examinations were associated with low physical risk, some imaging procedures involved exposure to ionising radiation. The examinations could also identify previously unknown abnormalities, including incidental findings of uncertain clinical significance, potentially leading to psychological distress, further investigations, and medical interventions. The additional procedures relevant to this thesis consisted of a questionnaire on women's health and a non-invasive assessment of cutaneous microvascular reactivity**.** These procedures entailed minimal risk, although arterial occlusion during the microvascular assessment could cause transient discomfort. As in all large-scale epidemiological studies, protection of participant privacy and secure handling of personal data were important ethical considerations.

RESULTS AND COMMENTS

Sustained physical activity

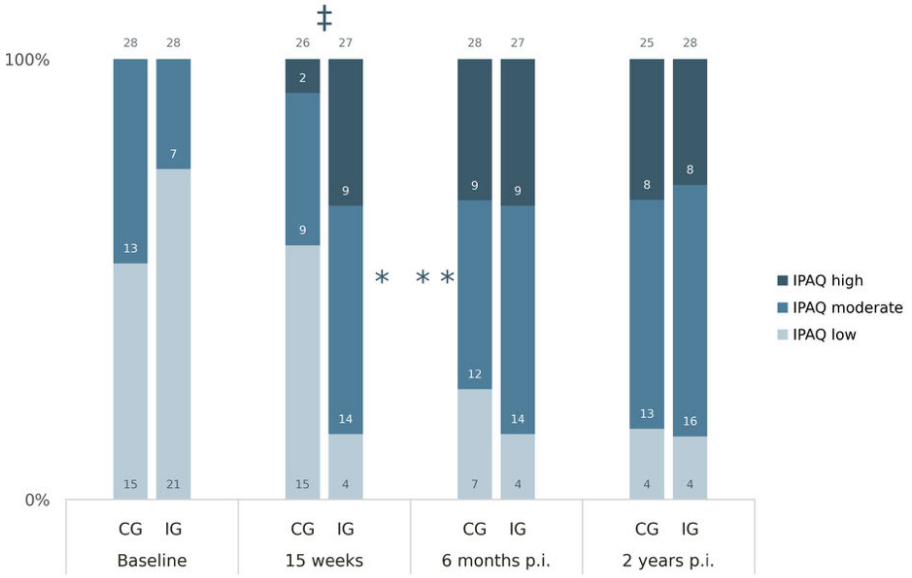

Figure 7. Self-reported physical activity levels during the 2-year follow-up

Stacked bars show the proportions of participants classified as having low, moderate, or high physical activity according to the International Physical Activity Questionnaire (IPAQ) in the intervention group (IG) and control group (CG). Numbers within the bars indicate the number of participants in each activity category; totals above the bars indicate the number with complete IPAQ data. The control group was offered resistance-training-related support after the 15-week assessment. *p<0.001 for change in the intervention group from baseline to 15 weeks. **p=0.016 for change in the control group from 15 weeks to 6 months post-intervention. ‡p<0.001 for the between-group difference at 15 weeks. p.i., post-intervention.

Self-reported physical activity increased during the intervention in the original intervention group and subsequently in the original control group (Figure 7). At baseline, 21 women (75%) in the intervention group and 15 (54%) in the control group were classified as having low IPAQ levels; no participants in either group had high IPAQ levels. At 15 weeks, four women in the intervention group and 15 in the control group had low IPAQ levels.

By the 2-year follow-up, four women in each group were classified as having low IPAQ levels.

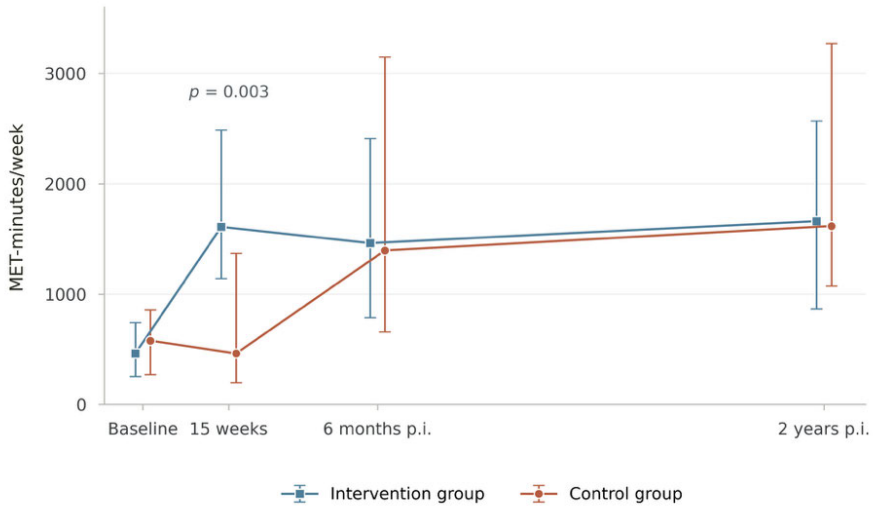

Figure 8. Self-reported total physical activity by intensity during the 2-year follow-up

Physical activity was assessed using the International Physical Activity Questionnaire. Total physical activity is expressed as metabolic equivalent of task (MET)-minutes per week. Points show medians, and error bars indicate the first and third quartiles. The intervention group included 29 participants at baseline and 28 at each subsequent assessment. The control group included 29 participants at baseline and 15 weeks, 28 at 6 months, and 25 at 2 years. The control group was offered resistance-training-related support after the 15-week assessment.The x-axis is proportional to elapsed time. Small horizontal offsets separate the groups. The p-value indicates the between-group comparison at 15 weeks. p.i., post-intervention.

Total physical activity, expressed as MET-minutes/week, followed a similar pattern (Figure 8). In the intervention group, median total physical activity exceeded baseline at every follow-up. Vigorous-intensity activity increased from 0 to 680 MET-minutes/week during the intervention ($p<0.01$) and remained at similar levels at 6 months and 2 years. Walking, moderate-intensity activity, and vigorous-intensity activity increased from baseline to 2 years in both groups. Between-group differences in IPAQ level, total physical

activity, vigorous-intensity activity, and MVPA were statistically significant at 15 weeks, but no statistically significant between-group differences were observed at baseline, 6 months, or 2 years.

The increase in physical activity was therefore sustained for 2 years in the original intervention group, supporting the hypothesis concerning long-term physical activity within this group. The subsequent increase in the original control group followed an introduction to resistance training, provision of a training diary, and temporary gym access. Both groups also received repeated emails about the health benefits of physical activity. This sequence suggests that both supervised training and more limited support may help initiate sustained behavioural change. However, the absence of an untreated comparator after week 15 prevents attribution of the long-term increases to individual components of the support or to the original randomised allocation.

The findings are consistent with evidence that behavioural interventions can produce sustained increases in physical activity. A systematic review and meta-analysis of studies with objective activity measurements and follow-up of at least 24 months found small increases in MVPA that persisted for up to 4 years [171]. The present results suggest that sustained increases may also be achievable among postmenopausal women with VMS and initially low physical activity, although activity was self-reported.

An accompanying qualitative study offers insight into how motivation may have developed during the intervention [179]. Although relief of bothersome VMS was initially an important reason for starting resistance training, women subsequently described enjoyment, improved well-being, and perceived health benefits as reasons for continuing. Professional support and accountability also facilitated participation. These findings suggest that reasons for exercising may broaden beyond symptom relief as women gain experience of training. They provide a plausible context for the sustained activity without establishing which components of the intervention produced it.

Gonadotrophin concentrations

At baseline, LH concentrations did not differ between the randomised groups, whereas FSH concentrations were higher in the control group than in the intervention group. In the PP analysis, LH decreased in the compliant intervention group but increased in the control group, resulting in a statistically significant between-group difference in change during the 15-week intervention period ($p = 0.028$). No statistically significant between-group difference in LH change was observed in the ITT analysis. No statistically significant between-group difference was observed for FSH (Figure 9).

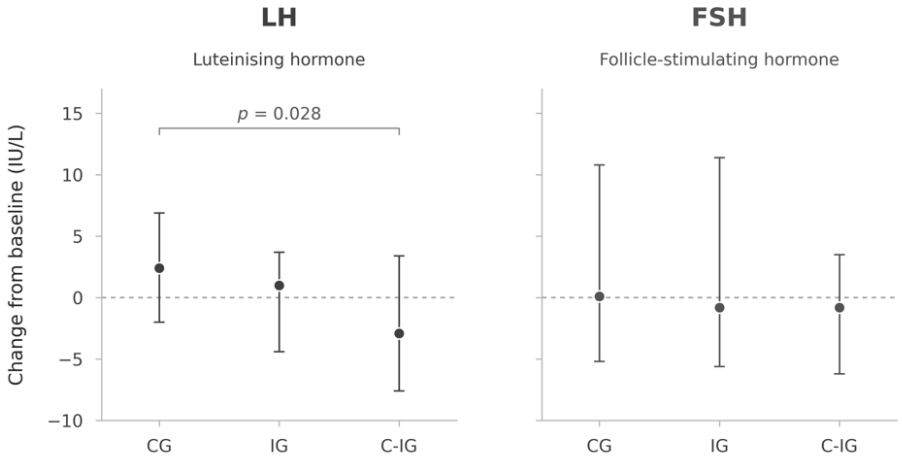

Figure 9. Changes in plasma LH and FSH concentrations after 15 weeks of resistance training

Points show median changes from baseline to 15 weeks, with first and third quartiles. Negative values indicate decreases. Green represents LH and blue FSH. CG, control group; IG, full intervention group; C-IG, participants averaging ≥2 training sessions/week. The bracket marks the LH comparison between C-IG and CG (p = 0.028, Mann–Whitney U test). LH change did not differ significantly between IG and CG. No FSH group differences were statistically significant.

Among intervention participants whose VMS frequency decreased by at least 50%, neither LH nor FSH changed significantly. Changes in gonadotrophin concentrations were also not associated with changes in VMS. The LH

response thus suggests a possible effect on neuroendocrine regulation but does not establish a mechanism for symptom improvement. Its restriction to the PP analysis is important: compliance was determined after randomisation, and the comparison may partly reflect characteristics of women who completed sufficient training. The finding therefore warrants confirmation in further randomised studies.

KNDy neurons provide a plausible link between the LH response and thermoregulation because they contribute both to the GnRH pulse generator and to pathways involved in heat dissipation. In mice, activation of arcuate Kiss1 neurons produced cutaneous vasodilatation and a fall in core body temperature, with a greater response after ovariectomy [35]. These experiments establish a thermoregulatory role for this neuronal network, but whether resistance training modifies its activity in postmenopausal women remains unknown.

The differing findings for LH and FSH may be compatible with altered GnRH pulsatility. LH secretion closely reflects pulsatile GnRH release, whereas FSH is additionally regulated by inhibins, activins, and follistatin [180]. NK3R antagonists also reduce LH secretion and suppress LH pulsatility, with less consistent effects on FSH. This pharmacological evidence supports the biological plausibility of a KNDy–GnRH pathway, but it does not show that training acts through the same mechanism. Moreover, a statistically significant result for LH and a non-significant result for FSH do not establish a selective effect on LH, particularly given the baseline imbalance in FSH.
Evidence from functional hypothalamic amenorrhoea demonstrates that exercise-related physiological conditions can alter this hormonal axis. In previously untrained women, strenuous exercise frequently disrupted ovulatory function, particularly when accompanied by weight loss [181]. Eumenorrhoeic runners have also shown lower LH pulse frequency, pulse amplitude, and integrated secretion than sedentary controls [182]. These findings support the responsiveness of hypothalamic regulation to exercise-related stimuli, although high training loads and low energy availability differ substantially from the resistance-training intervention studied here.

Endogenous opioid signalling is one possible mediator. Exercise may increase β-endorphin activity [183], and opioid antagonism has increased LH secretion and restored ovulatory activity in women with hypothalamic amenorrhoea [55, 184]. Resistance training might therefore influence KNDy or GnRH signalling through β-endorphin, dynorphin, or other neuromodulators. However, opioid activity, KNDy activity, and GnRH pulsatility were not measured, so the observed LH response provides only indirect evidence for these proposed pathways.

Studies of placebo analgesia have demonstrated involvement of endogenous opioid signalling [185, 186]. Given the role of endogenous opioids in KNDy signalling, placebo-related activation of these pathways might also influence VMS. This overlap could contribute to the pronounced placebo response observed in VMS trials, although this mechanism remains hypothetical.

Microvascular function

The study population comprised 1072 women aged 50–64 years with valid OxyP measurements. Their mean age was 57.7 years (SD 4.3), mean BMI was 26.4 kg/m² (SD 4.7), and mean OxyP was 84.1% (SD 4.2). Diabetes was present in 6.0% of participants, and 10.0% were current smokers.

Physical activity and sedentary time

OxyP differed across tertiles of MVPA. Women in the intermediate and highest tertiles had higher OxyP than women in the lowest tertile, whereas the intermediate and highest tertiles did not differ (Figure 10). These differences remained after multivariable adjustment. OxyP did not differ across tertiles of sedentary time.

The MVPA findings support the hypothesis that more physically active women would have higher OxyP. The pattern was characterised by a difference between the least active group and both more active groups, rather than progressively higher OxyP across all three tertiles. This does not establish a threshold or a dose–response relationship, and whether increasing MVPA would improve OxyP remains to be determined.

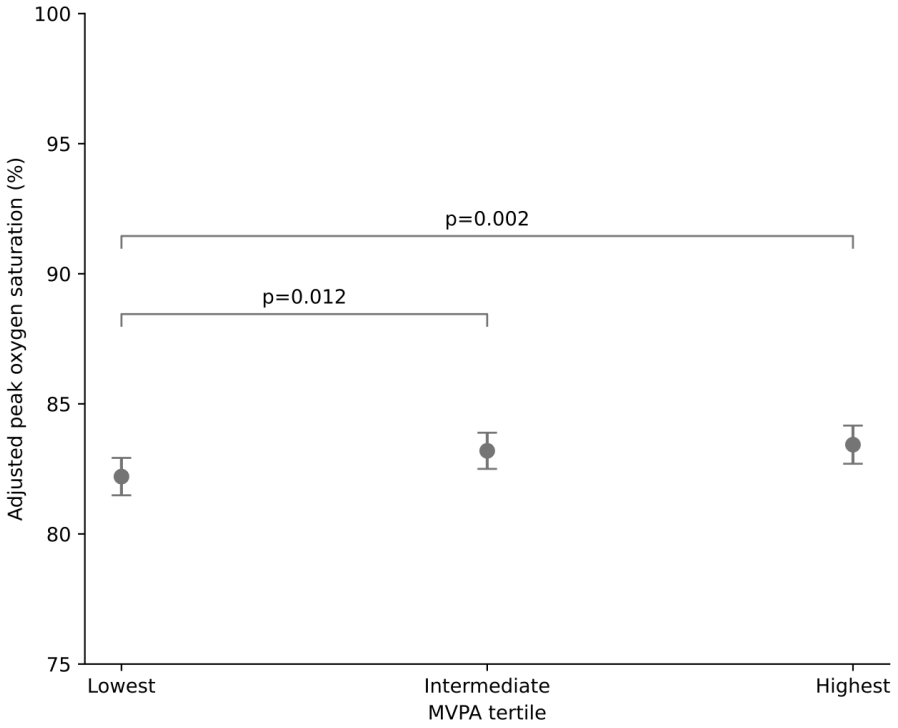

Figure 10. OxyP across tertiles of accelerometer-measured MVPA

Points represent estimated marginal means of OxyP adjusted for age, BMI, smoking, and diabetes; error bars indicate 95% CIs. Pairwise comparisons were adjusted for multiple comparisons using the Bonferroni method. OxyP was higher in the intermediate and highest MVPA tertiles than in the lowest tertile, whereas the intermediate and highest tertiles did not differ. MVPA, moderate-to-vigorous physical activity; OxyP, peak oxygen saturation following release of arterial occlusion.

The findings are consistent with previous exercise research. Endurance training improved cutaneous post-occlusive reactive hyperaemia in postmenopausal women [153], and a systematic review and meta-analysis found an overall benefit of exercise training for cutaneous microvascular reactivity [151]. The present results complement this evidence by demonstrating differences according to habitual MVPA in a large population-based sample of midlife women. Comparisons nevertheless require attention to the outcome measured: OxyP represents peak haemoglobin oxygen

saturation during reperfusion and is distinct from the perfusion-based measures used in many previous studies. Earlier SCAPIS analyses linked lower OxyP to conventional cardiovascular risk factors and greater subclinical atherosclerotic burden [105, 177], providing context for the differences observed here.

The absence of OxyP differences across sedentary-time tertiles contrasts with experimental evidence that uninterrupted sitting can acutely impair lower-limb microvascular responses [187, 188]. Accumulated sedentary time and forearm reactivity may capture different aspects of vascular physiology from acute responses to prolonged sitting. Similarly, the Maastricht Study found no relationship between sedentary time and skin microvascular function, although higher physical activity was related to greater heat-induced skin hyperaemia among participants with type 2 diabetes [189]. The present findings therefore do not exclude effects of uninterrupted sitting or effects in other vascular beds.

Menopausal status, menopausal hormone therapy use, and vasomotor symptom frequency

Table 3. Peak oxygen saturation according to menopausal status, current menopausal hormone therapy use, and VMS frequency

Exposure	n	OxyP (%), mean (SD)	Unadjusted p
Menopausal status			0.307
Premenopausal	147	84.5 (4.5)	
Postmenopausal	820	84.1 (4.1)	
MHT use			0.472
Never	820	84.1 (4.1)	
Current	93	83.8 (4.5)	
VMS frequency			0.055
None	433	84.0 (3.9)	
Less than daily	215	83.9 (4.6)	
Daily	158	84.8 (3.6)	

OxyP values are presented as mean (SD). The analysis of menopausal status included women who had never used MHT. The analysis of MHT use included postmenopausal women, and the analysis of VMS frequency included postmenopausal women who had never used MHT. Unadjusted p values were obtained using independent-samples t tests for menopausal status and MHT use and one-way analysis of variance

for VMS frequency. MHT, menopausal hormone therapy; OxyP, peak oxygen saturation following release of arterial occlusion; VMS, vasomotor symptoms.

OxyP did not differ between groups defined by menopausal status or current MHT use, or across groups defined by VMS frequency, in the unadjusted analyses (Table 3). The results remained unchanged after adjustment for age, BMI, smoking, and diabetes.

The findings did not support the hypotheses of higher OxyP before menopause or among current MHT users. This differs from evidence of lower conduit-artery flow-mediated dilatation across successive stages of the menopausal transition [190]. Findings from the cutaneous microcirculation have been less consistent: MHT users showed greater pharmacologically stimulated vasodilatory responses in a cross-sectional study, whereas intervention studies found no improvement after six months of MHT or short-term oestradiol administration [191-193]. Differences in vascular beds, hormonal regimens, participant characteristics, and physiological stimuli may explain some of this variation. The present results concern post-occlusive oxygen saturation and should not be generalised to all aspects of vascular function.

The VMS findings likewise need to be considered in relation to the vascular response assessed. Frequent or severe VMS have been linked to lower conduit-artery flow-mediated dilatation [194, 195], whereas enhanced cutaneous vasodilatory responses have been reported among women with hot flushes [196, 197]. Iliodromiti and colleagues found greater cutaneous vasodilatation but a lower digital reactive hyperaemia index in the same women [197]. These divergent findings indicate that VMS may relate differently to different vascular beds and responses. Here, groups were defined by symptom frequency during the preceding 2 weeks; the results do not address VMS severity, duration, or cumulative exposure.

Leukocyte telomere length

The between-group difference in LTL change from baseline to 15 weeks was not statistically significant. Among 50 women with paired measurements, the baseline-adjusted difference was 0.72 kb (95% CI −0.85 to 2.30; p=0.361). Positive values indicated a greater increase in the resistance training group. Additional adjustment for age yielded a similar estimate.

The comparison from baseline to two years included 33 women. LTL change did not differ significantly between the original randomisation groups after adjustment for baseline LTL (difference −0.17 kb, 95% CI −1.98 to 1.64; p=0.848).

Both groups were pooled to examine physical activity during follow-up in relation to LTL change. Among 32 women, mean MVPA was not significantly associated with LTL change from 15 weeks to two years. Adjusting for 15-week LTL, the estimate was 0.018 kb per 60 min/week higher MVPA (95% CI −0.107 to 0.144; p=0.770). Additional age adjustment and sensitivity analyses did not alter the interpretation of these findings. The adjusted estimates are summarised in Table 4.

Table 4. Adjusted group differences and the relation between physical activity and LTL change.

Analysis	n	Estimate (95% CI)	p-value
Between-group difference, baseline-15 weeks, kb	50	0.72 (−0.85 to 2.30)	0.361
Between-group difference, baseline-2 years, kb	33	−0.17 (−1.98 to 1.64)	0.848
MVPA and LTL change, 15 weeks-2 years, kb per 60 min/week	32	0.018 (−0.107 to 0.144)	0.770

Group differences represent resistance training minus control, adjusted for baseline LTL. The MVPA analysis pooled both groups and adjusted for LTL at 15 weeks. Mean MVPA was calculated from available six-month and two-year assessments. LTL is expressed in kilobase pairs per chromosome end. CI, confidence interval; LTL, leucocyte telomere length; MVPA, moderate-to-vigorous physical activity.

The hypothesis that resistance training would increase LTL or attenuate its shortening was not supported by the randomised comparison. However, the

wide confidence interval included both lower and higher LTL change in the intervention group relative to controls. The findings therefore do not establish an absence of effect. No separate sample-size calculation was performed for LTL, and the small analysis population limited precision.

Previous observational findings on telomere length and physical activity have been mixed. Longer LTL has been reported in habitually active postmenopausal women and in individuals with greater lower-extremity muscle strength [156][198]. However, a systematic review and meta-analysis found no overall association between physical activity and LTL, with substantial heterogeneity across studies [157]. Differences in health and lifestyle, together with possible reverse causation, complicate interpretation of these observational relationships.

Our follow-up analysis did not support the hypothesis that higher physical activity would be associated with longer LTL. However, it assessed change over time rather than cross-sectional differences in LTL. Self-reported activity at two assessments may not adequately capture exposure throughout the interval. The small sample also limited the ability to distinguish an association from random variation.

Intervention evidence raises the possibility that responses differ by exercise modality. In a randomised trial, endurance and high-intensity interval training increased telomerase activity and LTL, whereas resistance training did not [158]. Telomerase activity also increased acutely after endurance exercise but not after resistance exercise [158]. These results suggest that particular metabolic or haemodynamic stimuli may be relevant, although differences in training duration, intensity, participants, and laboratory methods limit comparisons between trials.

A recent umbrella review and meta-analysis reported a small-to-moderate positive overall effect of exercise on telomere length [199]. However, findings varied across studies, and the review included different populations, exercise modalities, and both randomised and non-randomised trials. These pooled findings do not establish an effect of resistance training in postmenopausal women.

The 2-year findings were exploratory because only a subset of participants provided samples and both original groups could undertake resistance training after week 15. The hypothesis that women originally allocated to resistance training would have longer LTL at two years was not supported. This comparison concerns the original allocations, rather than continued resistance training versus an untreated control condition. It cannot determine whether sustained resistance training affects LTL over two years.

DISCUSSION

Methodological considerations

Strengths

A central strength of this thesis is the combination of intervention-based and population-based study designs. The randomised trial enabled assessment of changes during a structured resistance training intervention, whereas the SCAPIS-based study provided complementary evidence from a large sample of midlife women. The longitudinal and cross-sectional approaches addressed different aspects of physical activity and exercise, including sustained physical activity and biological outcomes related to neuroendocrine regulation, microvascular reactivity, and cellular ageing.

For comparisons during the initial 15-week intervention, randomisation provided protection against confounding, although chance baseline imbalances could still arise in a trial of this size. The resistance training programme was standardised and progressive, with individually prescribed loads based on repetition maximum. One session per week was supervised by a physiotherapist, and attendance and training loads were documented. These procedures supported intervention fidelity while allowing most sessions to be performed independently in an ordinary gym setting. Participation in the intervention was monitored using both individual training logbooks and the gym's electronic card-registration system. The initial use of lower loads followed by gradual progression was intended to improve tolerability and reduce the risk of injury and may have contributed to the low dropout rate. However, the contribution of individual programme components to intervention completion cannot be determined.

The study of microvascular function was based on a large population-based sample of women randomly invited from the population registry, rather than recruited according to symptoms, physical activity, or healthcare use. Standardised SCAPIS phenotyping provided information on cardiovascular risk factors and enabled prespecified multivariable adjustment. Accelerometry provided objective measurements of MVPA and sedentary time, reducing recall and reporting bias. Cutaneous microvascular reactivity was assessed non-invasively using a standardised occlusion–release protocol,

making physiological assessment feasible in a large study population. The supplementary women's health questionnaire enabled the comparison of OxyP across groups defined by menopausal status, systemic MHT use, and recent VMS frequency within the same cohort.

Limitations

The nature of the intervention precluded blinding participants to group allocation, and the STYRKA trial was therefore conducted as an open trial. This limitation is particularly relevant for self-reported outcomes such as VMS and physical activity. Participants in the intervention group received structured exercise, professional attention, social interaction, and an expectation of improvement. Participants in the initially untreated control group knew that they were not receiving the active intervention. This differs from blinded pharmacological trials, in which participants in both groups may expect active treatment. VMS show a pronounced placebo response in such trials; hot flush frequency decreased by a mean of 58% among placebo recipients according to a Cochrane review [29]. Between-group differences in an open exercise trial may therefore reflect both physiological and contextual effects. Changes in the untreated group may also reflect natural symptom variation, regression to the mean, and secular changes. The open design is less likely to have introduced direct measurement bias in the laboratory outcomes, although contextual effects may have influenced participants' behaviour and thereby indirectly affected these outcomes.

Randomisation reduced confounding for intention-to-treat comparisons during the initial 15-week intervention but did not confer the same protection on all analyses in this thesis. The compliant subgroup was defined after randomisation. Comparisons involving this subgroup may therefore be influenced by characteristics associated with compliance. Similarly, the randomised groups no longer represented distinct intervention exposures after 15 weeks. The original control group was offered an introduction to resistance training and temporary gym access, and both groups could subsequently change their physical activity. The two-year analyses therefore describe trajectories according to original allocation but cannot estimate a sustained effect of the original randomised intervention. Furthermore, they cannot

determine which components of the exercise support contributed to the sustained increase in physical activity. More detailed information on exercise modality, frequency, and intensity throughout follow-up, together with repeated accelerometer measurements, would have strengthened the assessment of sustained behavioural change.

The temporal ordering of MVPA and OxyP could not be determined in the cross-sectional analysis. Higher physical activity may improve microvascular reactivity, but women with better vascular function or general health may also be more able to engage in physical activity. Residual confounding by health-related, behavioural, or socioeconomic factors may remain despite multivariable adjustment. The observed differences in OxyP across MVPA groups therefore cannot establish causality or its direction.

Measurement considerations

The STYRKA study relied on self-reported physical activity measured using the IPAQ. Questionnaire-based measurements are susceptible to recall error and social desirability bias and may overestimate activity. IPAQ may also be less sensitive to individual changes over time than to differences in activity at the population level. Furthermore, it did not identify which exercise modalities participants performed during follow-up. Consequently**,** the findings demonstrate a sustained increase in self-reported physical activity but not sustained participation in resistance training. Accelerometry could have provided a more objective assessment of general movement behaviour, although commonly used accelerometers also capture resistance training and some non-ambulatory activities poorly.

The study of gonadotrophins used single fasting measurements of LH and FSH at baseline and 15 weeks. Gonadotrophin secretion is pulsatile, and a single measurement does not characterise pulse frequency or amplitude. Repeated sampling would have provided a more direct assessment of changes in hypothalamic–pituitary regulation. Neither KNDy neuron activity nor endogenous opioid activity was measured. The proposed involvement of these pathways is therefore based on indirect interpretation rather than direct mechanistic evidence.

Menopausal status, MHT use, and VMS were self-reported in SCAPIS and may have been misclassified. The women's health questionnaire was pilot-tested for face validity and showed good test–retest stability, but it was not formally validated against clinical assessments or medical records. In the present analyses, VMS assessment was restricted to symptom frequency during the preceding two weeks and did not capture symptom severity, duration, age at onset, or cumulative exposure. Women reporting no current VMS may previously have experienced symptoms. The MHT analyses classified participants according to current or never use but did not account for differences in formulation, dose, administration route, or treatment duration.

Accelerometry reduced reporting bias but did not capture water-based activities and may have underestimated cycling, resistance exercise, and activities involving limited movement of the hip. Women who regularly performed these activities may therefore have been classified into lower MVPA tertiles despite substantial exercise exposure.

Furthermore, sedentary time was defined from movement counts and may therefore have included periods of stationary standing. The sedentary measure included only time accumulated in uninterrupted bouts of at least 20 minutes. It therefore did not capture total sedentary time or shorter bouts of sedentary behaviour. Thus, the absence of OxyP differences across sedentary tertiles does not exclude differences related to how sedentary time was accumulated. MVPA and sedentary time were categorised into tertiles. This facilitated interpretation but reduced information and limited detailed assessment of dose–response relationships or clinically meaningful thresholds.

The EPOS system provides simultaneous non-invasive measurements of microvascular oxygen saturation and perfusion [103, 104]. OxyP, however, represents a specific aspect of post-occlusive cutaneous microvascular reactivity. Oxygen saturation and perfusion provide distinct information and should not be considered interchangeable. It should not be interpreted as a comprehensive measure of endothelial function or as a direct measurement of

coronary microvascular function. Comparisons with previous studies are limited because vascular beds, physiological stimuli, and measurement techniques differ.

The standardised occlusion protocol, preceding rest, and controlled room temperature helped reduce measurement variability. Nevertheless, the probe samples a limited skin region, and local microvascular heterogeneity may influence the measurements. As a peak value, OxyP also does not capture the timing or duration of the response. Despite standardisation, residual medication effects and deviations from restrictions concerning food, caffeine, nicotine, and alcohol may have contributed to measurement variability. Withholding medication before examination may not eliminate its effects, particularly for drugs with long elimination half-lives.

In the study of telomere length, qPCR provided an estimate of mean LTL across a heterogeneous population of circulating leucocytes. Measurements may be influenced by DNA quality, laboratory variation, amplification efficiency, and changes in leucocyte composition. The qPCR method is fundamentally comparative, and conversion to kilobase pairs depended on calibration against a reference sample with a known mean telomere length. The resulting values should therefore be interpreted as calibrated estimates rather than directly measured absolute telomere lengths. Analysis in triplicate and placement of samples from the same participant within the same batch reduced, but did not eliminate, measurement variability. Moreover, the biological and clinical significance of short-term changes in mean LTL remains uncertain.

Selection and generalisability

Participants in STYRKA actively volunteered for an exercise trial and may have been more motivated to change their behaviour than postmenopausal women in general. They had low physical activity at baseline, experienced frequent moderate-to-severe VMS, and were sufficiently healthy to perform resistance training. The findings may therefore not be generalisable to physically active or asymptomatic women, women with substantial comorbidity, or those less motivated to exercise. The compliant subgroup

constituted a further selected group of women able and willing to complete the prescribed training.

Only a small subset of the original cohort provided LTL samples at two years. The extension was introduced after recruitment had begun, and the earliest participants were not eligible for this assessment. Selective participation may therefore have influenced the observed long-term LTL trajectory.

Although prospective SCAPIS participants were randomly selected from the population registry, participation was lower in socioeconomically disadvantaged groups, and participants were generally healthier than the background population. The present sample was restricted to women aged 50–64 years from the Linköping study site who had valid OxyP measurements and sufficiently complete menopausal data. Exclusion of women with uncertain menopausal status or former MHT use increased the specificity of the comparisons but reduced representativeness. The smaller numbers of premenopausal women, current MHT users, and women with daily VMS also reduced precision for these comparisons.

Statistical considerations

The STYRKA trial was powered for its original VMS outcome rather than for the secondary outcomes examined in this thesis. The relatively small sample limited statistical power and the precision of the estimated effects, as well as limited the ability to perform subgroup analyses. A non-significant result therefore does not exclude smaller effects on gonadotrophins or LTL. Conversely, findings from secondary and per-protocol analyses should be regarded as exploratory.

Intention-to-treat analyses estimate the effect of allocation to an intervention strategy and preserve the benefits of randomisation. Per-protocol analyses describe outcomes among participants who met the prespecified compliance criterion but do not preserve the protection against confounding provided by randomisation**.** The LH finding was observed in the compliant subgroup and should therefore not be interpreted as equivalent to an intention-to-treat treatment effect. The baseline imbalance in FSH concentrations, which may

have arisen by chance in this relatively small trial, further complicates the interpretation of changes in this outcome.

The LTL analyses compared changes between groups using linear regression with adjustment for baseline LTL. Each analysis required paired measurements, reducing the sample and potentially introducing selection bias. Wide confidence intervals limited conclusions about intervention effects. Bootstrap analyses, exclusion of influential observations, and alternative definitions of follow-up MVPA did not materially alter the interpretation. These checks did not resolve the uncertainty arising from the small samples. The pooled MVPA analysis was observational and remained susceptible to residual confounding. The two-year group comparison was exploratory because the sample was small and both groups had access to resistance training after week 15.

Several prespecified group comparisons were evaluated using sequential adjustment models in the SCAPIS-based analysis. Bonferroni correction was applied to post-hoc comparisons when the overall test was statistically significant, but multiple analyses still increase the possibility of chance findings. Categorisation of continuous movement variables into tertiles may also have reduced statistical power. Although the models included important cardiovascular risk factors, residual confounding cannot be excluded. Missing data were not imputed, and complete-case analyses may introduce selection bias if missingness is related to both the group-defining variables and OxyP.

Findings in relation to the thesis objectives

Evaluation of the specific hypotheses

- **The hypothesis that an increase in physical activity following a resistance training intervention could be sustained for two years was supported within the original intervention group.** Physical activity increased during the 15-week intervention and remained above baseline after two years. However, physical activity also increased in the original control group after resistance-training-related support was offered, and the groups did not differ at long-term follow-up.
- **The hypothesis that resistance training would reduce circulating gonadotrophin concentrations was partially supported.** Among participants who met the prespecified compliance criterion, LH concentrations decreased relative to the control group during the 15-week intervention, whereas the corresponding difference in FSH was not statistically significant. No statistically significant between-group differences were observed in the ITT analysis.
- **The hypothesis that premenopausal women would have higher OxyP than postmenopausal women was not supported.** Among women who had never used MHT, OxyP did not differ between premenopausal and postmenopausal women, either before or after multivariable adjustment.
- **The hypothesis that postmenopausal current MHT users would have higher OxyP than never users was not supported.** OxyP did not differ between current and never users of MHT, either before or after multivariable adjustment.
- **The hypothesis that OxyP would differ across groups defined by VMS frequency was not supported.** OxyP did not differ among postmenopausal women with no current, less than daily, or daily VMS.
- **The hypothesis that higher physical activity and lower sedentary behaviour would be accompanied by higher OxyP was partially supported.** Women in the intermediate and highest MVPA tertiles had higher OxyP than women in the lowest tertile, and these

differences remained after multivariable adjustment. However, OxyP did not differ across sedentary time tertiles.

- **The hypothesis that resistance training would increase LTL or attenuate its shortening over 15 weeks was not supported.** LTL change did not differ significantly between the intervention and control groups after adjustment for baseline LTL.
- **The hypothesis that higher physical activity during follow-up would be associated with longer LTL at two years was not supported.** Mean MVPA during follow-up was not significantly related to LTL change from 15 weeks to two years, accounting for 15-week LTL.
- **The hypothesis that women randomised to resistance training would have longer LTL at two years than controls was not supported.** LTL change from baseline did not differ significantly between the original randomisation groups after adjustment for baseline LTL.

Synthesis in relation to the general aim

The general aim of this thesis was to investigate the role of physical activity in climacteric women, with a particular focus on whether increased physical activity could be sustained and on neuroendocrine function, microvascular reactivity, and cellular ageing. Taken together, the findings show that physical activity is relevant across several, but not all, of these dimensions. Most importantly, increased self-reported physical activity was sustained for two years after exposure to resistance training and related behavioural support. The increase observed in both original randomised groups suggests that even relatively limited support, including an introduction to resistance training and temporary access to exercise facilities, may help initiate sustained behavioural change. This is clinically relevant in postmenopausal women with VMS and low physical activity, for whom establishing regular exercise may itself provide substantial long-term health benefits.

The findings also provide two notable biological signals. The LH response among participants meeting the compliance criterion extends previous exercise research in postmenopausal women beyond symptom outcomes and raises the possibility that resistance training influences neuroendocrine

regulation when enough training is completed. Furthermore, the consistently higher OxyP among women in the two higher MVPA tertiles, including after adjustment for major cardiovascular risk factors, identifies habitual MVPA as an important characteristic of women with greater cutaneous microvascular reactivity. These findings provide a foundation for mechanistic and interventional studies examining neuroendocrine and vascular responses to exercise.

By contrast, LTL change did not differ significantly between the original randomisation groups at either follow-up and was not significantly associated with physical activity during follow-up. The small samples and wide confidence intervals limit conclusions about these findings. Overall, the thesis supports resistance training with behavioural support as a feasible means of promoting sustained physical activity in climacteric women and identifies promising neuroendocrine and microvascular findings that merit further investigation.

Future perspectives

The findings of this thesis raise several questions that I would find particularly interesting to pursue. First, do resistance training and aerobic exercise differ in their effects on VMS? Direct comparisons with MHT and placebo could establish the relative magnitude and broader health effects of these interventions.

Combining exercise with an NK3R antagonist may produce greater reductions in VMS than either treatment alone. If the interventions act through partly different pathways, their combined effect might approach that of MHT. A related question is whether NK3R antagonism, alone or combined with exercise, could reduce VMS in men receiving androgen-deprivation therapy for prostate cancer.

The observed LH response raises a more specific mechanistic question. Does endogenous opioid signalling contribute to the effect of exercise on VMS? If an opioid antagonist such as naltrexone attenuated the symptom reduction, this would support an opioid-mediated mechanism. Concurrent changes in LH could provide complementary evidence concerning hypothalamic regulation.

The absence of differences in OxyP across VMS groups does not exclude dynamic vascular differences during a hot flush. Do women with VMS respond differently to heat than women without symptoms? Studies during standardised heating and spontaneous flushes could identify differences in the threshold, magnitude, or recovery of the cutaneous microvascular response. Intervention studies could also determine whether increasing MVPA improves OxyP.

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APPENDIX

Supplementary questionnaire on women's health in SCAPIS

Extra frågor till kvinnor i SCAPIS

1. Har din mens upphört eller delvis upphört? (oavsett orsak)
□ Ja, upphört (ingen mens senaste året). Vid vilken ålder hade du din sista mens (ange ungefärligt om du inte minns säkert)? Vid___års ålder.
□ Ja, delvis upphört (mens gjort uppehåll minst 3 månader i sträck senaste året).
□ Nej. *Gå direkt till fråga 6.*

2. Om din mens upphört på grund av gynekologisk operation, vid vilken ålder skedde operationen? Vid___års ålder.

3. Om din mens upphört på grund av hormonbehandling eller hormonella preventivmedel, vid vilken ålder skedde detta? Vid___års ålder.

4. Om din mens upphört eller delvis upphört på grund av klimakteriet, var/är klimakteriet förknippat med klimakterierelaterade besvär?

Värmevallningar och/eller nattliga svettningar:
□ Ja, besvär i hög grad. Om ja, hur länge varade dina besvär? Mellan___och___års ålder eller pågår fortfarande sedan ___års ålder.
□ Ja, besvär i ganska hög grad. Om ja, hur länge varade dina besvär? Mellan___och___års ålder eller pågår fortfarande sedan ___års ålder.
□ Ja, besvär i mindre grad. Om ja, hur länge varade dina besvär? Mellan___och___års ålder eller pågår fortfarande sedan ___års ålder.
□ Nej, inga besvär.

Andra typer av klimakterierelaterade besvär (flera alternativ möjliga om olika typer av besvär):
□ Ja, besvär i hög grad. Om ja, vilken/vilka typer av besvär? _____
Hur länge varade dina besvär? Mellan___och___års ålder eller pågår fortfarande sedan ___års ålder.
□ Ja, besvär i ganska hög grad. Om ja, vilken/vilka typer av besvär? _____
Hur länge varade dina besvär? Mellan___och___års ålder eller pågår fortfarande sedan ___års ålder.
□ Ja, besvär i mindre grad. Om ja, vilken/vilka typer av besvär? _____
Hur länge varade dina besvär? Mellan___och___års ålder eller pågår fortfarande sedan ___års ålder.
□ Nej, inga besvär.

5. Har du under de senaste 2 veckorna haft klimakteriebesvär i form av värmevallningar och/eller svettningar?
□ Ja, flera gånger/dag
□ Ja, enstaka gånger/dag
□ Ja, ett par gånger/vecka
□ Ja, enstaka besvär
□ Nej, inga besvär

6. Använder/använde du någon receptbelagd östrogeninnehållande behandling för klimakteriebesvär i form av värmevallningar och/eller nattliga svettningar?
Tabletter:
□ Ja, använder nu. Vid vilken ålder började behandlingen? Vid___års ålder.
□ Ja, har tidigare använt. När skedde behandlingen? Mellan___och___års ålder.
□ Nej, har aldrig använt.

Plåster eller gel:
□ Ja, använder nu. Vid vilken ålder började behandlingen? Vid___års ålder.
□ Ja, har tidigare använt. När skedde behandlingen? Mellan___och___års ålder.
□ Nej, har aldrig använt.

7. Använder/använde du någon receptbelagd östrogeninnehållande behandling för klimakteriebesvär i form av torra slemhinnor?
Tabletter:
□ Ja, använder nu. Vid vilken ålder började behandlingen? Vid___års ålder.
□ Ja, har tidigare använt. När skedde behandlingen? Mellan___och___års ålder.
□ Nej, har aldrig använt.

Plåster eller gel:
□ Ja, använder nu. Vid vilken ålder började behandlingen? Vid___års ålder.
□ Ja, har tidigare använt. När skedde behandlingen? Mellan___och___års ålder.
□ Nej, har aldrig använt.

Vagitorier (slidpiller), vaginal kräm/gel eller vaginalinlägg:
□ Ja, använder nu. Vid vilken ålder började behandlingen? Vid___års ålder.
□ Ja, har tidigare använt. När skedde behandlingen? Mellan___och___års ålder.
□ Nej, har aldrig använt.

8. Använder/använde du något naturläkemedel, till exempel s.k. fytoöstrogener som behandling för klimakteriebesvär?
□ Ja, använder nu. Vid vilken ålder började behandlingen? Vid___års ålder.
□ Ja, har tidigare använt. När skedde behandlingen? Mellan___och___års ålder.
□ Nej, har aldrig använt.

9. Har du någon gång under livet använt hormonella preventivmedel? (flera alternativ möjliga)
□ Ja, p-piller. Ungefär under hur lång period sammanlagt? Totalt___år.
□ Ja, minipiller. Ungefär under hur lång period sammanlagt? Totalt___år.
□ Ja, annan typ av hormonellt preventivmedel (p-plåster, p-ring, p-stav, p-spruta, hormonspiral eller kopparspiral). Ungefär under hur lång period sammanlagt? Totalt___år.
□ Nej, har inte använt.

10. Har du någon period i livet haft besvär med premenstruella spänningar (PMS) såsom nedstämdhet, humörsvängningar, lätt att gråta, bröstspänningar, svullnadskänsla i kroppen?
□ Ja, besvär i hög grad.
□ Ja, besvär i ganska hög grad.
□ Ja, besvär i mindre grad.
□ Nej, inga besvär.
Hur länge varade dina PMS-besvär? Mellan___och___års ålder eller pågår fortfarande sedan ___års ålder.

Tack för din medverkan!

Papers

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