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REVIEW-SYMPOSIUM

The time is now: regular exercise maintains vascular health in ageing women

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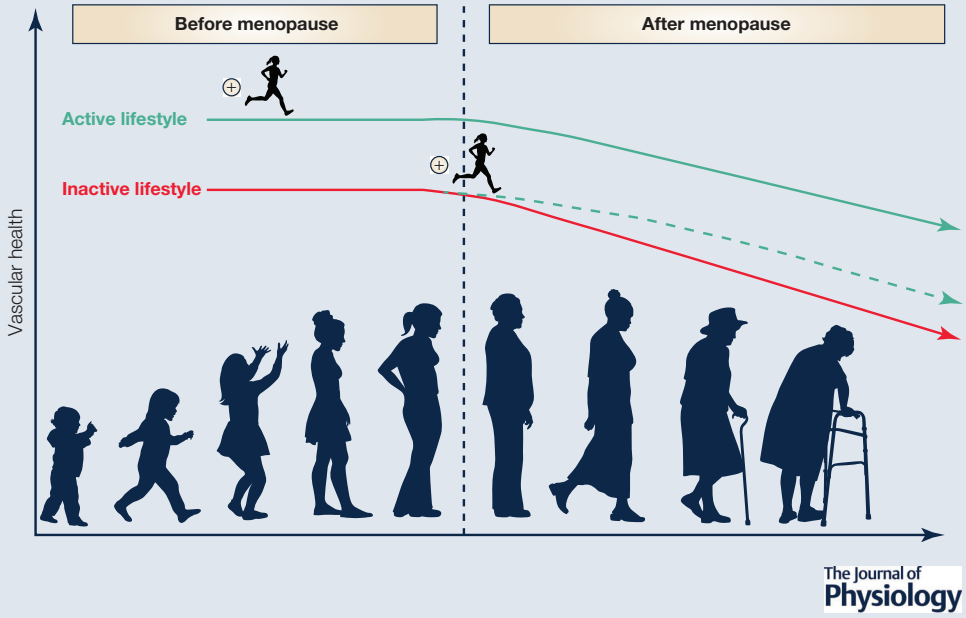

Abstract Although ageing impairs cardiovascular health in both men and women, the timeline is different between the sexes. This is at least partially attributed to the loss of oestrogen in women at midlife, in connection with menopause. Oestrogen has protective effects on the cardiovascular system, and menopause consequently leads to a rapid and significant

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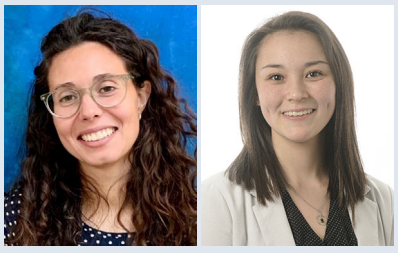

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decline in cardiovascular health. Notably, oestrogen interacts with its nuclear and membrane receptors leading to changes in proteins of importance for cardiovascular health. Skeletal muscle activity, which affects the expression of many of the same proteins as oestrogen, could potentially counteract the loss of oestrogen at menopause. The hypothesis that exercise can counteract the loss of oestrogen has been explored in several recent studies. It has been found that regular physical activity opposes the detrimental effects not only of ageing, but also of the menopausal transition, on cardiovascular health. Although, vascular benefits can be gained at all ages, initiating physical activity at or soon after menopause may be more effective than at a later time point in life. Intuitively, it is easier to prevent decrements than attempting to regain lost vascular health. This idea is supported by evidence at the molecular level, suggesting that exercise-induced activation of the oestrogen-related receptor-α pathway is more effective soon after menopause compared to later. Together, although a decline in cardiovascular health due to chronological ageing cannot be completely prevented, a physically active lifestyle mitigates age-related cardiovascular impairments. Importantly, regular physical activity through life should always be addressed as the biological norm.

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Abstract figure legend Compared to a physically inactive lifestyle, lifelong participation in physical activity protects against the development of vascular disease. However, ageing and menopause, irrespective of physical activity status, have inevitable, negative effects on vascular health. Importantly, if regular exercise is initiated around the menopausal transition, the vascular consequences of ageing and menopause can be, at least partially, mitigated.

Introduction

The female sex hormone oestrogen exerts a myriad of positive effects on the vascular system, which can explain the lower vascular disease risk in premenopausal women compared to age-matched men (Parker et al. 2010). However, with menopause and the cessation of oestrogen production, vascular function is impaired and the risk of developing vascular disease dramatically increases (Parker et al. 2010). Arterial blood pressure, which is a reliable functional marker of overall vascular health (Fuchs & Whelton, 2020), increases after menopause. Accordingly, the prevalence of hypertension is greater in post- compared to premenopausal women and increases with years/time after menopause (Lima et al. 2012). The rise in arterial blood pressure is the result of several changes in the vascular architecture as well as the regulation of vascular tone (Moreau et al. 2012; Nyberg et al. 2014). Structurally, larger arteries become atherosclerotic and less compliant whereas at the microvascular level, rarefaction may occur (Landers-Ramos & Prior, 2018). Functionally, the regulation of peripheral vascular resistance is impaired in part due to enhanced sympathetic vasoconstriction and enhanced levels of circulating vasoconstrictors, such as thromboxane A_2, combined with reduced formation and/or efficiency of peripheral vasodilators (Hearon & Dinenno, 2016). Although these changes occur with chronological age *per se*, it should be emphasized that they will occur to a markedly greater extent with physically *inactive* ageing, rather than physically *active* ageing, and that, biologically, being active through life is the norm for humans. In women, maximal oxygen uptake ($\dot{V}_{O_2\,max}$) decreases significantly with advancing age, where we have observed a significant negative linear relationship between $\dot{V}_{O_2\,max}$ and age ($R^2 = 0.60$ and P-value ≤ 0.0001). Interestingly, our findings also show that women with a lifelong active lifestyle break this relationship by, to some degree, maintaining $\dot{V}_{O_2\,max}$ despite advancing age (Fig. 1). In line with this notion, countless studies have shown that regular physical activity retains vascular function throughout life and decreases the risk of vascular events in both men and women (Nystoriak & Bhatnagar, 2018; Seals et al. 2019).

Nevertheless, there is a catch for ageing women. A series of studies have shown that menopause may reduce or even omit the positive effects of physical activity on vascular health (Moreau & Hildreth, 2014; Moreau et al. 2013; Santos-Parker et al. 2017). However, there are also studies showing that exercise training can induce positive adaptations in postmenopausal women (Lundberg Slingsby et al. 2017; Nyberg et al. 2016). The studies reporting clear beneficial effects have involved women who are recently postmenopausal (i.e. <5 years since last menstrual bleeding) and/or have applied more vigorous training protocols. Based on the combined findings in previous studies, we propose in this symposium review that: (1) benefits from exercise may be more rapidly

gained at or soon after the menopausal transition rather than later in menopause, at which time it may take longer to reach the same benefits; (2) higher intensity exercise may be more effective at compensating for the loss of oestrogen in menopause; and most importantly, (3) exercise has an array of beneficial effects on an individual's health and well-being and a physically active lifestyle should always be advised irrespective of age. This review discusses the current literature investigating the capacity for physical activity to improve vascular health before and at menopause as well as beyond. We highlight several aspects related to vascular health – blood pressure, regulation of vascular resistance, markers of blood clot formation and skeletal muscle angiogenesis – and propose a potential underlying molecular mechanism for the exercise timing aspect. Each section briefly discusses the effect of ageing, the effect of physical activity, differences between sexes, and what is known regarding the timing of exercise training in women. It should be noted that there are limited available data in postmenopausal women for some of the adaptations included in this review, and conclusions in these areas should therefore be interpreted as preliminary.

Oestrogen receptors, oestrogen-related receptor-α, menopause and exercise training

Oestrogen elicits its protective effects on the vasculature via two main oestrogen receptor-mediated pathways in endothelial cells: (1) genomic regulation, involving the activation of oestrogen response elements (ERE) to alter protein expression, e.g. increased endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), and superoxide dismutase 2 (SOD2), and (2) non-genomic regulation, through direct activation of signalling cascades, e.g. increased phosphorylation of eNOS on Ser-1177 (Gliemann & Hellsten, 2019; Menazza & Murphy, 2016). Additionally, oestrogen can post-translationally modify proteins via G protein-coupled oestrogen receptor 1 (GPER) activation

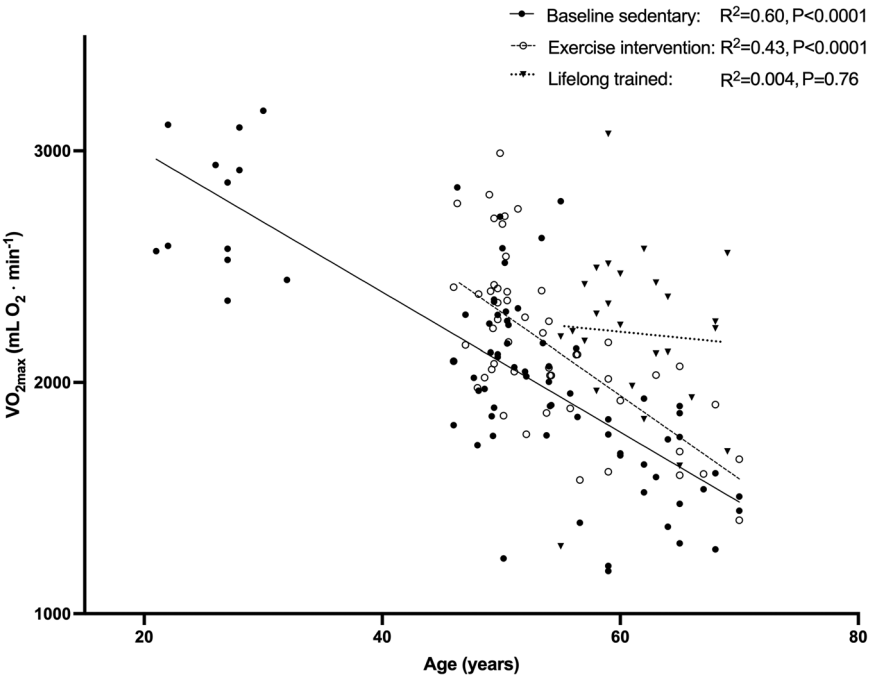

Figure 1. Correlations between Correlations between $\dot{V}_{O_2\,max}$ and age in sedentary women and after an exercise intervention
Linear correlations between $\dot{V}_{O_2\,max}$ and age in sedentary women before (filled circles, $n = 76$; unpublished data, Gliemann, Rytter et al. 2020; Hoier et al. 2021; Nyberg et al. 2016) and after an exercise intervention (8–12 weeks, open circles, $n = 52$; Hoier et al. 2021; Nyberg et al. 2016) as well as a group of lifelong trained women (triangles, $n = 26$; Gliemann, Rytter et al. 2020), both moderately and highly trained (moderately: 2–4 h of low to moderate intensity exercise and 1 h of high-intensity exercise per week; highly: more than 4 h of moderate- and high-intensity exercise per week). There was a significant relationship between $\dot{V}_{O_2\,max}$ and age in sedentary women ($R^2 = 0.6039$ and $P < 0.0001$) and after the exercise intervention ($R^2 = 0.4339$ and $P < 0.0001$). There was no linear relationship between $\dot{V}_{O_2\,max}$ and age in the lifelong trained group ($R^2 = 0.004$ and $P = 0.764$). The data are combined from unpublished data and previous published data (see references). Women included in the 8–12 weeks training intervention were tested before and after the intervention and are therefore included in both the sedentary and exercise intervention group.

(Prossnitz & Barton, 2011). For example, GPER activation can turn on signalling cascades that promote eNOS activation (Fredette et al. 2018). The combined effects of oestrogen and its receptors enhance both eNOS activity and nitric oxide (NO) bioavailability, which is critical for vasodilatation and shear stress-induced angiogenesis but also for promoting an anti-inflammatory phenotype in the vasculature, by quenching circulating reactive oxygen species (ROS) and inhibiting leukocyte adhesion (Förstermann & Münzel, 2006). This, in combination with the antioxidant properties of oestrogen and its effect on upregulation of SOD2 and catalase, makes the role of oestrogen in limiting oxidative stress and inflammation substantial (Ighodaro & Akinloye, 2018; Ribon-Demars et al. 2019). Lastly, oestrogen may also modulate capillary growth in skeletal muscle by its influence on NO bioavailability and VEGF expression (Hyder et al. 2000). Capillary growth in skeletal muscle has important implications for health, as capillaries facilitate the transport and delivery of oxygen and nutrients to target tissues and can ease the stress generated from hypoxic environments, as seen in disease states such as hypertension or diabetes (Kim & Byzova, 2014). Taken together, oestrogen-promoted proteins are clearly critical for the maintenance of endothelial health and vascular function (Fig. 2).

Interestingly, previous rodent (Novensà et al. 2011) and human (Novella, Heras et al. 2012) studies have suggested that oestrogen has the potential to elicit both pro- and anti-inflammatory responses in the vasculature, where the dominant phenotype is influenced by the number of years after menopause. Oestrogen receptor α (ERα) is purported to be predominantly responsible for exerting the anti-inflammatory effects, while oestrogen receptor β (ERβ) has been suggested to be related to a pro-inflammatory profile (Novella, Heras et al. 2012). The loss of oestrogen associated with the menopausal transition has been proposed to decrease ERα protein expression, thereby increasing the ratio of ERβ:ERα, and potentially favouring a pro-inflammatory phenotype (Novensà et al. 2011; Park et al. 2017), although a greater depth of research unearthing the nuances of the relationship between ERα and ERβ, particularly in humans, is still warranted.

Importantly, regular physical activity can mimic some of the effects of endogenous oestrogen by activating the orphan nuclear receptor oestrogen-related receptor α (ERRα). *In vitro* studies in skeletal muscle cell cultures have shown that muscle contraction activates ERE in the absence of oestrogen, and that the effect is mitogen-activated protein kinase-dependent, indicating activation via ERRα (Wiik et al. 2009). This, combined with observations that ERα protein expression decreases after menopause (Novensà et al. 2011; Park et al. 2017) and exercise training upregulates ERRα protein expression in recent postmenopausal but not premenopausal women (Nyberg et al. 2017), could suggest that after menopause exercise-induced signalling through the ERRα pathway may become more important. Notably, the ERRα pathway is coupled to the peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) pathway and promotes the production of several of the same key proteins related to vascular health as oestrogen, e.g. eNOS and SOD2, as well as mitochondrial biogenesis (Craige et al. 2016; Perry et al. 2014). Cumulatively, before menopause oestrogenic effects can be achieved by both endogenous oestrogen and regular physical activity, whereas after menopause only the ERRα pathway remains. The previously mentioned finding that ERRα protein content increased significantly in postmenopausal but not premenopausal women following a period of exercise training could suggest that ERRα compensates for the menopause-related loss of ERE activation (Nyberg et al. 2017). Another potentially critical aspect is that this pathway may lose its efficacy with time, as ERRα protein content declines with years after menopause in sedentary women (Gliemann & Hellsten, 2019). Together, this evidence highlights the importance of exercise training, and the potential reliance on the ERRα pathway, in postmenopausal women for the preservation of vascular health (Gliemann & Hellsten (2019) (Fig. 2).

Arterial blood pressure

Chronically elevated arterial pressure is a strong predictor of vascular disease and a major cause of mortality worldwide (Fuchs & Whelton, 2020). A sustained increase in blood pressure not only influences cardiac work, but also contributes to systemic vascular changes, which in turn may elevate blood pressure further and increase the risk of organ damage (Mennuni et al. 2014) and thrombosis (Faraco & Iadecola, 2013). In Europe, reports indicate that about 50% of men and 39% of women between 35 and 74 years of age have clinically elevated blood pressure (Wolf-Maier et al. 2003). Interestingly, men show a relatively steady rise in arterial pressure with age, whereas women have largely unaltered blood pressure until menopause and then commonly present a rather rapid rise in arterial pressure (Barton & Meyer, 2009; Staessen et al. 1997). This accelerated rise is, at least in part, due to the hormonal changes occurring. Previous studies have suggested that the oestrogen receptor GPER is the main receptor responsible for the potent eNOS-mediated vasodilatory effects of oestrogen, and although the evidence is limited, lower GPER protein content has been associated with hypertension in postmenopausal women (Liu et al. 2018). Yet, GPER expression does not appear to be directly related to the loss of oestrogen at menopause, as the same study

reported similar GPER protein expression in pre- and postmenopausal women (Liu et al. 2018). However, there are several known causes of hypertension, and there are clearly multiple factors involved in the accelerated rise in blood pressure after menopause.

As with most vascular changes, the rise in arterial pressure depends to a large extent on lifestyle, where an active lifestyle significantly attenuates the rise. Accordingly, a comparison of arterial blood pressure between endurance trained and sedentary

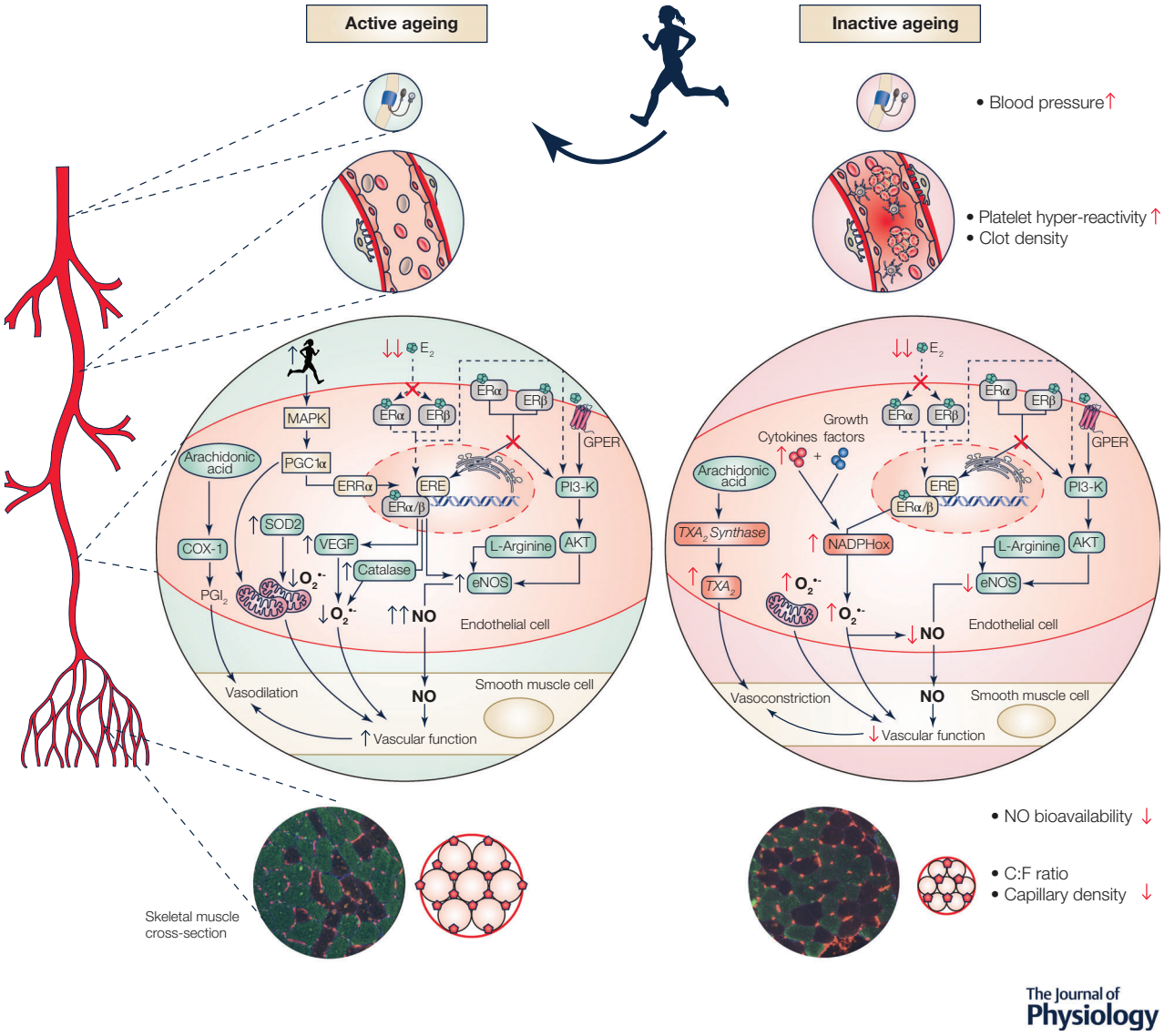

Figure 2. Outline of differences in vascular health with active ageing *versus* inactive ageing in women
Active ageing is characterized by a conservation of vascular health both in the macrovasculature and microvasculature and subsequently a slowed deterioration when compared to the typical trajectory observed in sedentary ageing women. Inactive ageing in women is characterized by a rapid rise in arterial blood pressure following menopause as well as an increase in thrombotic risk, platelet reactivity, and inflammation. Concurrently, reactive oxygen species, specifically superoxide anions ($O_2^{\bullet-}$), accumulate and lead to lower nitric oxide (NO) bioavailability, which can impair endothelial function. Moreover, inactive ageing is characterized by low capillary density and capillary-to-fibre (C:F) ratio. Importantly, initiating an exercise intervention can restore vascular health, although timing of the initiation of the interventions are of great importance. COX-1, cyclooxygenase-1; E_2, oestradiol; eNOS, endothelial nitric oxide synthase; ERα, oestrogen receptor α; ERβ, oestrogen receptor β; ERE, oestrogen response element; ERRα, oestrogen-related receptor α; GPER, G protein-coupled oestrogen receptor 1; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; PI3-K, phosphoinositide 3-kinase; SOD2, superoxide dismutase 2; TXA_2, thromboxane A_2; VEGF, vascular endothelial growth factor.

postmenopausal women revealed that well-trained women had significantly lower systolic pressure than sedentary women (Santos-Parker et al. 2017). Another cross-sectional study demonstrated that women with a lifelong moderately active lifestyle with ∼2–4 h of low- to moderate-intensity exercise and ∼1 h high-intensity training per week had lower blood pressure levels than sedentary women (Gliemann, Rytter et al. 2020). These data suggest that a moderately active lifestyle is sufficient to oppose the age-induced increase in blood pressure. In fact, the mean arterial blood pressure in the moderately active group was not statistically different from that of a group of very active postmenopausal women, who performed more than 4 h of moderate- to high-intensity exercise per week (Gliemann, Rytter et al. 2020).

Several studies have also demonstrated that as little as 2–3 months of regular physical activity can lower arterial blood pressure in postmenopausal women. Some of these studies have utilized intense aerobic interval cycling (Hoier et al. 2021; Nyberg et al. 2016) and others high-intensity running, e.g. floorball and interval running (Gliemann, Tamariz-Ellemann et al. 2020; Nyberg et al. 2014), and have found that these intensive exercise modalities produce beneficial reductions in arterial blood pressure (Fig. 3). Conversely, findings with low- to moderate-intensity training (e.g. walking and Nordic walking) are somewhat more divergent, with some studies showing a lowering of blood pressure (Cebula et al. 2020; di Blasio et al. 2012) and no effect in others (Moreau et al. 2013; Pierce et al. 2011). Combined, existing data from midlife and older women suggest that regular physical activity of moderate- to high-intensity is effective in opposing the age-related increase in arterial blood pressure in women, whereas low- to moderate-intensity exercise may be somewhat less effective.

Vascular function

Conduit artery function. Flow-mediated dilatation (FMD) is a non-invasive method that measures changes in artery diameter with ultrasound Doppler in response to increased flow following reactive hyperaemia (Hoier et al. 2021). FMD is used as an indicator of overall vascular health, and epidemiological studies suggest a relationship between the brachial artery FMD response and the risk for vascular events (Green et al. 2011), proving its clear validity as a clinically relevant marker. However, it may be pointed out that FMD evaluates endothelial function of conduit arteries, which have no functional role in the regulation of blood flow or peripheral resistance.

In both men and women there is a gradual decline in the brachial artery FMD response with ageing but in women there is a more prominent decline at midlife, specifically occurring after the menopausal transition (Holder et al. 2019). This suggests that the FMD response in women may be accelerated by the menopause-associated changes in sex hormone levels (Moreau et al. 2012). Sex-specific effects have been reported regarding the impact of physical activity on the FMD response in middle-aged men and postmenopausal women. Interestingly, both longitudinal

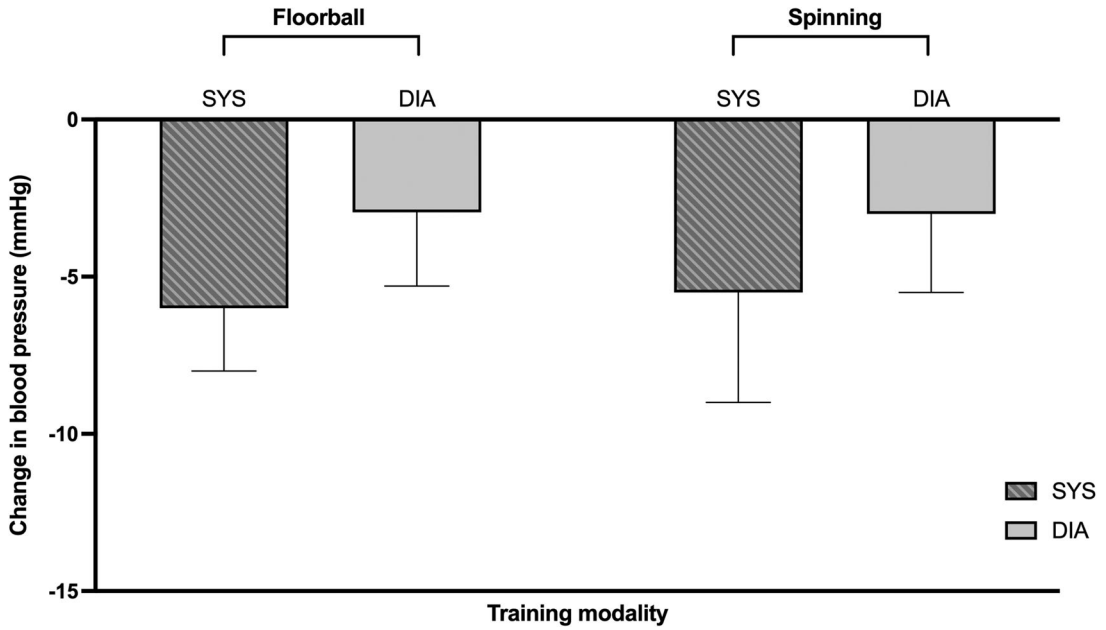

Figure 3. Reductions in clinical systolic and diastolic blood pressure by exercise intervention in sedentary postmenopausal women
Data adapted and combined from: Gunnarsson et al. (2020), Hoier et al. (2021) and Nyberg et al. (2014, 2016). Floorball, $n = 18$; spinning, $n = 27$.

and cross-sectional comparisons have shown no effect of moderate-intensity walking training on FMD in postmenopausal women, despite a significant improvement with similar training in age-matched men (Pierce et al. 2011). In a follow-up study, the same research group observed that men and women only attained the same magnitude of improvement in FMD after training when postmenopausal women were provided a combination of walking training and oestrogen replacement therapy (Moreau et al. 2013).

Traditionally, FMD is assessed in the brachial artery even when the exercise training modality involves predominantly lower body exercise (e.g. cycling). Thus, it could be argued that an improvement in FMD could be achieved more easily in the trained limbs of the women (e.g. the legs if performing cycling training). However, Hoier et al. (2021) showed no improvement in popliteal artery FMD after 8 weeks of aerobic cycle training in women more than 10 years after menopause. Thus, at least in late postmenopausal women, changes in FMD appear difficult to achieve, regardless of type of exercise and site of measurement, but evidence also suggests that regular exercise with a combination of oestrogen supplementation (Moreau et al. 2013) can lead to an improved FMD response.

Skeletal muscle microvascular function. Intra-arterial infusions of vasoactive substances are used to evaluate endothelial function in the smaller arterioles, which play an essential role in the regulation of peripheral resistance and therefore blood pressure and local blood flow. The method employs simultaneous measurements of blood flow by Doppler ultrasound technology and intra-arterial blood pressure, enabling the calculation of vascular resistance or conductance. Although this method is useful for assessing microvascular function, the invasive nature of this method limits the use to smaller scale studies. Accordingly, non-invasive protocols, such as FMD, are preferably used for larger cohorts.

Intra-arterial infusion of acetylcholine in combination with a smooth muscle stimulating vasodilators, such as sodium nitroprusside (NO donor) or the prostacyclin (PGI_2) analogue epoprostenol, enables investigation of the ability of the endothelium in the smaller resistance arterioles to produce and secrete vasodilators and induce vasodilatation in the vascular smooth muscle cells (Nyberg et al. 2016). The vasodilator response to arterial infusion of acetylcholine decreases as a function of age with a negative linear correlation ($R^2 = 0.74$) in healthy normotensive subjects (Taddei et al. 1995). Evidence of a decline in microvascular function with the menopausal transition has been provided by Nyberg and colleagues (2016), whereby a ∼14–41% lower response to acetylcholine and epoprostenol was observed in recent postmenopausal compared to late premenopausal women of similar age (age gap of ∼4 years). These findings suggest that the decline in vascular function is already present in the early stages of menopause and continues with advancing age.

Exercise training has consistently been shown to prevent and recover age-related declines in vascular function in older men (Desouza et al. 2000; Taddei et al. 2001). However, in older women, the exercise-induced improvements in vascular function are inconsistent and less convincing (Gliemann, Tamariz-Ellemann et al. 2020; Nyberg et al. 2016). The opposing findings regarding the effect of exercise on microvascular function in older women have been suggested to be the result of discrepancies between age and/or postmenopausal stage and/or the exercise intervention. For example, in women just around the menopausal transition (∼50 years, <3 years after menopause), 8 or 12 weeks of aerobic cycle exercise has been shown to improve the vasodilator response to acetylcholine by as much as ∼20% (Nyberg et al., 2012, 2016). Conversely, in older women further from the menopausal transition (∼60 years of age, >5 years after menopause), the improvements in vascular function with a period of high-intensity floorball training were not statistically significant (Gliemann, Tamariz-Ellemann et al. 2020). However, cross-sectional studies show that older women (∼60 years of age) with a lifelong highly active lifestyle, exhibit a greater responsiveness in leg vascular conductance to intra-arterial infusions of acetylcholine, compared to sedentary and moderately active older women (Gliemann, Rytter et al. 2020). Enhanced activation of the ERRα–PGC1α pathway may underpin a potential mechanism for the increase in vascular function with physical activity (Gliemann & Hellsten, 2019). Notably, in the study by Nyberg et al. (2017), ERRα was upregulated by ∼60% after a 12-week training intervention. This is further supported by the cross-sectional data from Gliemann, Rytter et al. (2020), where they found a significant, albeit limited, positive correlation between the skeletal muscle protein expression of ERRα and expression of eNOS, as well as between ERRα content in muscle samples and the vascular response to acetylcholine.

In summary, women with a lifelong physically active lifestyle exhibit preserved microvascular function, compared to lifelong sedentary women, and the initiation of exercise after the menopausal transition has the potential to improve microvascular function, at least when initiated within the first years after the menopausal transition.

Platelet reactivity and blood clot formation

The human body is constantly forming and breaking down blood clots. However, when an imbalance between

clot formation and degradation occurs, large blood clots that are not sufficiently degraded may trigger severe thrombotic events including arterial thrombosis. One in four people worldwide dies from a thrombotic event, and the risk of thrombosis dramatically increases after the age of 60 (Wendelboe & Raskob, 2016). Although men typically have a two-fold higher risk of thrombotic events compared to women (Roach et al. 2014), menopause significantly increases a woman's risk of thrombosis (Canonico et al. 2014). Available data on indicators of thrombosis are discussed below in relation to menopause and exercise training.

Platelet reactivity. Platelet reactivity, consisting of platelet activation, adhesion and aggregation, is critical for blood clot formation (Periayah et al. 2017). Platelet reactivity can be assessed by exposing platelet-rich plasma to known concentrations of platelet agonists. Although premenopausal females have lower vascular disease risk than males (Parker et al. 2010), it is well-established that at all ages, females have higher platelet counts and are more responsive to agonist-induced aggregation than males (Sabetta et al. 2022). Moreover, a growing body of evidence demonstrates that even with healthy ageing, platelets become hyper-reactive and are less sensitive to inhibition, although the exact mechanisms remain to be elucidated (le Blanc & Lordkipanidzé, 2019). Interestingly, menopause poses an additional challenge to optimal platelet function, as oestrogen is an important positive regulator of the production of NO and PGI_2, which are established inhibitors of platelet activation (Novella, Dantas et al. 2012). Consequently, it has been hypothesized that the loss of oestrogen associated with menopause may promote an imbalance in platelet reactivity, favouring platelet hyper-reactivity, which subsequently increases the risk of a thrombotic event (Bray, 2007). However, existing literature is both scarce and conflicting. A pilot study by Singla et al. (2013) showed no significant difference between late premenopausal and recent postmenopausal women in platelet reactivity induced by several agonists. Conversely, Slingsby et al. (2017) demonstrated higher resting platelet reactivity in response to the agonist thrombin receptor activator peptide 6 (TRAP-6) in early postmenopausal women compared to late premenopausal women, suggesting a basal state of platelet hyper-reactivity. Accordingly, the existing evidence is currently unclear regarding the impact of menopause on basal platelet reactivity.

Recent evidence suggests that exercise training significantly improves platelet function, as well-trained men have significantly reduced basal platelet reactivity and improved platelet sensitivity to prostacyclin compared to untrained and moderately trained men (Lundberg Slingsby et al. 2018). Additionally, as mentioned above, regular physical activity, via ERRα and ERE activation, stimulates several of the same vascular protective pathways as oestrogen and may thus be an effective strategy to reduce platelet hyper-reactivity in post-menopausal women. Notably, exercise training has been shown to increase circulating NO (Esmail et al. 2011; Zaros et al. 2009) and PGI_2 (Gliemann, Tamariz-Ellemann et al. 2020) levels. Additionally, 3 months of high-intensity exercise training improved platelet PGI_2 sensitivity in both late pre- and early postmenopausal women (Lundberg Slingsby et al. 2017). Though preliminary, these findings may suggest that exercise training can be beneficial for improving platelet function. However, more studies are clearly required to validate these findings.

Blood clot microstructure. The relatively novel application of rheometry allows for the generation of *in vitro* blood clots from whole blood (Kaibara, 1996). The gel point occurs when the blood transitions from a visco-elastic fluid to a viscoelastic solid, marking the formation of the incipient blood clot (Evans et al. 2008). Fractal dimension (d_f), which is a quantitative measure of the incipient clot microstructure (i.e. density and strength), provides a clinically relevant marker of thrombotic risk. A higher d_f signifies a stronger and denser blood clot that is more difficult to degrade via fibrinolysis (Evans et al. 2008).

So far, fractal dimension has predominantly been utilized for investigating thrombogenicity in clinical populations (Lawrence et al. 2015), but this method is clearly useful for the assessment of changes in clot density and strength with healthy ageing and to elucidate whether exercise training can alter this parameter. Preliminary data from our laboratory shows that d_f is markedly higher in healthy, postmenopausal women compared to young, healthy women (Fig. 4). Importantly, very small changes in d_f reflect dramatic changes in normalized clot mass, whereby as little as a 0.02 increase in d_f signifies a $\sim$25% increase in clot mass (Sabra et al. 2017). Although not evaluated in our analysis, an estimation based on the work by Sabra et al. (2017) suggests that healthy, postmenopausal women form blood clots that have between 25% and 75% more mass than their young, healthy counterparts. Healthy ageing and menopause are associated with impairments to fibrinolysis as well as increases in platelet reactivity and plasma concentrations of coagulation factors (Bucciarelli & Mannucci, 2009). Moreover, the loss of oestrogen with menopause may exacerbate these age-related haemostatic changes (Meilahn et al. 1992) and previous work using a different methodology has demonstrated that post-menopausal women form denser *ex vivo* clots than pre-menopausal women (Piróg et al. 2016). Together, these

findings may explain our observation of a higher d_f in postmenopausal women compared to young women.

Capillarization and effect of age and menopause

In skeletal muscle, capillarization is crucial for the delivery of oxygen and nutrients (Egginton, 2009) and holds important implications for health, especially regarding glucose tolerance and insulin sensitivity (Akerstrom et al. 2014; Bonner et al. 2013). It is well-known that regular physical activity provides a potent stimulus that promotes an increase in skeletal muscle capillary growth in young, healthy individuals (Hoier et al. 2012). However, the influence of sex and ageing are less clearly understood. Studies investigating the impact of female hormones and menopause on skeletal muscle capillarization are rare, yet a recent meta-analysis showed clear sex-related differences in the capillary-to-fibre (C:F) ratio after a period of exercise training, whereby the increase in C:F ratio was 56% higher on average in males compared to females (Liu et al. 2022). Currently, the findings from studies on training-induced muscle capillary growth in postmenopausal women are inconsistent (Gavin et al. 2014; Gliemann et al. 2021; Gries et al. 2018; Olsen et al. 2020; Perez-Gomez et al. 2021). For example, Gavin et al. (2014) demonstrated an ∼20–25% increase in capillarization after 8 weeks of moderate-intensity training (heart rate equivalent to 65% of $\dot{V}_{O_2\,max}$) in middle-aged to older women, but Olsen et al. (2020) reported unaltered capillary growth after 8 weeks of high-intensity spinning training in women of similar age. Additionally, a cross-sectional study showed that life-long exercise-trained older men and women had same amount of capillarization as young exercise trained men and women performing the same number of training hours per week. However, old sedentary men and women had 20–90% lower capillarization (Gries et al. 2018). In an attempt to identify the role of oestrogen *versus* ageing on muscle capillary adaptations to training, Peréz-Goméz et al. (2021) assessed capillarization in late pre- and recent postmenopausal women of similar age (49 *vs.* 53 years of age) before and after 12 weeks of high-intensity spinning training. Similar increases in C:F ratio and capillary density of ∼6–13% were found in the two groups after training, suggesting that the hormonal change around menopause did not significantly influence the capacity for training-induced capillarization (Perez-Gomez et al. 2021). To what extent time/years after menopause influences training-induced capillarization has not been directly determined, but data from our laboratory provide an indication of a negative correlation between age and C:F ratio ($R^2 = 0.12$; $P = 0.007$) (Fig. 5). Interestingly, this relationship does not apply to lifelong-trained women (Fig. 5) (Gliemann et al. 2021). Although, the sample size is small, this latter finding suggests that lifelong training can help to maintain skeletal muscle capillarization with age. This notion is supported by Gliemann et al. (2021), who showed that a very high activity level throughout life is required for higher levels of skeletal muscle capillarization.

Taken together, although the data are somewhat sparse, it appears that capillary rarefaction occurs with age in sedentary postmenopausal women, but to a lesser extent in women who have conducted lifelong exercise training. A potential explanation for the more robust capillary rarefaction with sedentary ageing may be reduced endothelial cell proliferation as well as the level of VEGF (Olsen et al. 2020). However, VEGF increases after an 8-week training period in postmenopausal women (Olsen et al. 2020), and in the absence of oestrogen, ERRα may play a role in mediating this exercise-induced upregulation (Stein et al. 2009). Thus, longer training periods might be needed to induce skeletal muscle capillarization in postmenopausal women.

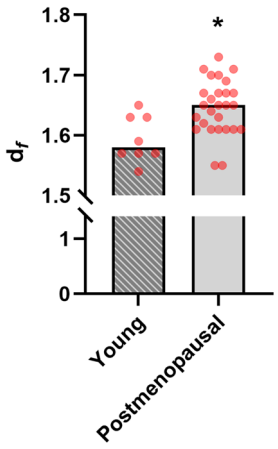

Figure 4. Fractal dimension analysis
Fractal dimension (d_f) analysis indicating increased mechanical strength and density of incipient blood clots in healthy postmenopausal women (n = 27; age: 58 ± 5 years; 8 ± 5 years after menopause; $\dot{V}_{O_2\,max}$: 27.6 ± 5.6 ml kg^{-1} min^{-1}) compared to young, healthy women (n = 8; age: 28 ± 2 years; $\dot{V}_{O_2\,max}$: 45.5 ± 2.4 ml kg^{-1} min^{-1}) (P = 0.005). All participants were fasted and avoided caffeine (24 h), strenuous exercise (48 h) and non-steroidal anti-inflammatory drugs (7 days). After the participants had rested in a supine position for 15 min, blood samples were drawn into vacutainers with no additive.

Perspective

In this review, we emphasize that regular physical activity is essential for healthy human ageing. However, it is interesting to consider that countries around the world have extremely divergent habits for participation in and adherence to regular physical activity, which is likely attributed to a multitude of factors including lifestyle,

values and accessibility. Importantly, many of the studies included in this review were conducted in Copenhagen, Denmark, a country that reports one of the highest levels of regular physical activity in the world. Conversely, only ∼40% of American adults and ∼10% of Japanese adults meet the physical activity recommendations (Sisson & Katzmarzyk, 2008). As highlighted in this review, a physically inactive lifestyle clearly contributes to what is commonly considered vascular ageing and accordingly, future studies should carefully consider the physical activity levels of participants when interpreting basal vascular data and the changes with ageing and physical activity.

Conclusion

In conclusion, the time to begin regular physical activity is now. A physically active lifestyle is imperative for minimizing declines in vascular health across the lifespan, and lifelong physically active older women display the best trajectory for vascular health (Fig. 2). However, if women have been sedentary until mid-life and onward, it is not too late to become active, although it appears that initiating regular and rigorous physical activity before the menopausal transition, rather than later in life, is likely more effective at mitigating the age-related impairments to vascular health.

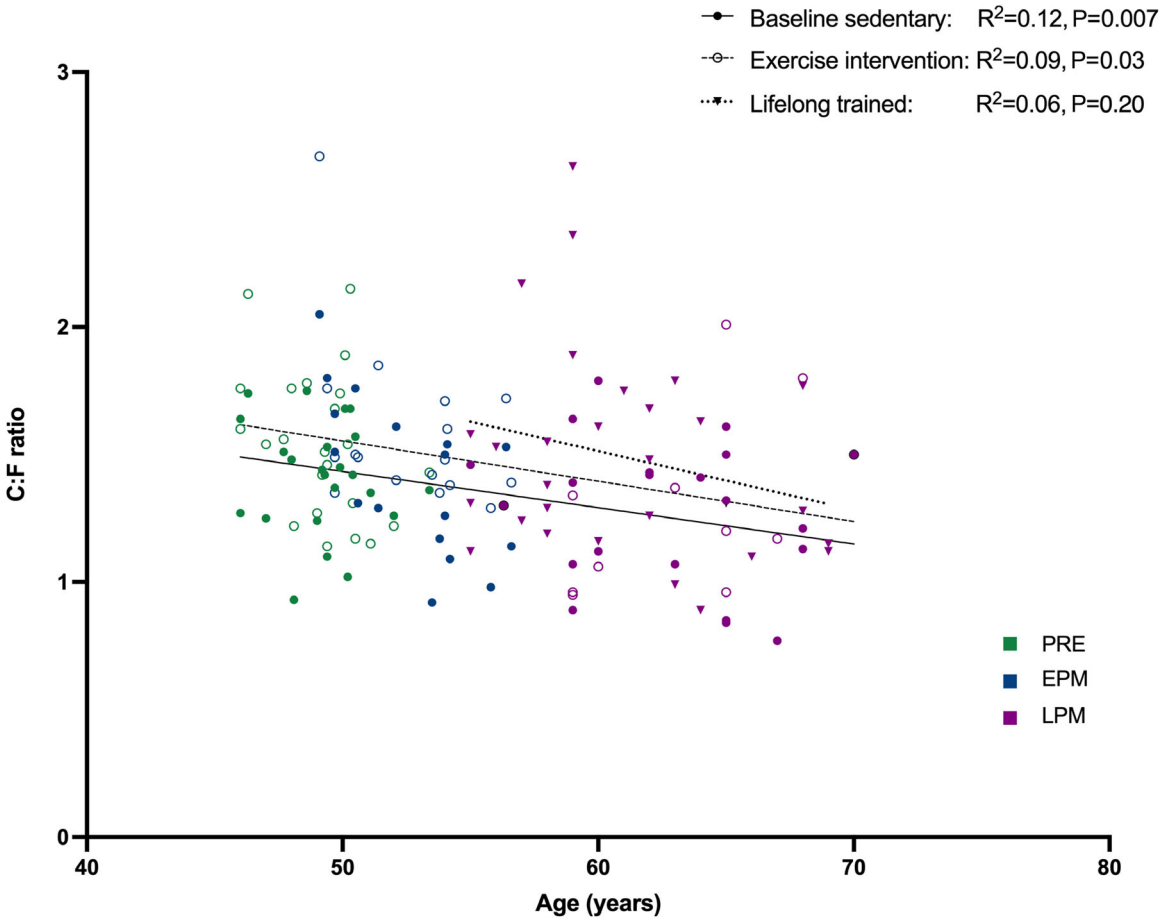

Figure 5. Linear correlations between capillary-to-fibre ratio and age
Linear correlations between capillary-to-fibre ratio (C:F ratio) and age, in sedentary women before (filled circles, $n = 61$; Gliemann et al. 2021; Olsen et al. 2020; Perez-Gomez et al. 2021) and after an exercise intervention (8–12 weeks, open circles, $n = 59$; Olsen et al. 2020; Perez-Gomez et al. 2021) as well as a group of lifelong trained women (triangles, $n = 29$; Gliemann et al. 2021), both moderately and highly trained (moderately: 2–4 h of low to moderate intensity exercise and 1 h of high-intensity exercise per week; highly: more than 4 h of moderate- and high-intensity exercise per week). There was a significant relationship between C:F ratio and age in sedentary women ($R^2 = 0.12$ and $P = 0.007$) and after the exercise intervention ($R^2 = 0.09$ and $P = 0.03$). There was no linear relationship between C:F ratio and age in the lifelong trained group ($R^2 = 0.06$ and $P = 0.204$). Data adapted and combined from previous published papers: Gliemann et al. (2021), Olsen et al. (2020) and Perez-Gomez et al. (2021).

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Additional information

Competing interests

The authors have no conflicts of interest to declare.

Author contributions

All authors were involved in drafting and designing the review as well as interpreting the findings. All authors contributed to the writing of the manuscript and the final version of the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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