The time is now: Regular exercise maintains vascular health in aging women

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

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Running title: Exercise training and vascular function in menopause

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Abstract

Although aging 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 estrogen in women at midlife, in connection with menopause. Estrogen has protective effects on the cardiovascular system, and menopause consequently leads to a rapid and significant decline in cardiovascular health. Notably, estrogen 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 estrogen, could
potentially counteract the loss of estrogen at menopause. The hypothesis that exercise can counteract the loss of estrogen has been explored in several recent studies. It has been found that regular physical activity opposes the detrimental effects not only of aging, but also 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 estrogen-related receptor alpha pathway is more effective soon after menopause compared to later. Together, although a decline in cardiovascular health due to chronological aging 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.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CD</td>
<td>Capillary Density</td>
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<td>C:F Ratio</td>
<td>Capillary to Fiber Ratio</td>
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<td>d_f</td>
<td>Fractal Dimension</td>
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<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthase</td>
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<td>ERα</td>
<td>Estrogen Receptor Alpha</td>
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<td>ERβ</td>
<td>Estrogen Receptor Beta</td>
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<td>ERE</td>
<td>Estrogen Response Element</td>
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<td>ERRα</td>
<td>Estrogen-Related Receptor Alpha</td>
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Introduction

The female sex hormone estrogen 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 estrogen 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<sub>2</sub> (TXA<sub>2</sub>), 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 aging, rather than physically active aging, and that, biologically, being active through life is the norm for humans. In women, maximal oxygen uptake (VO<sub>2max</sub>) decreases significantly with advancing age, where we have observed a significant negative linear relationship between VO<sub>2max</sub> and age (R<sup>2</sup>=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 VO<sub>2max</sub> 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 aging women. A series of studies have shown that menopause may reduce or even omit the positive effects of physical activity on vascular health (Moreau et al., 2013; Moreau & Hildreth, 2014; Santos-Parker et al., 2017). However, there are also studies showing that exercise training can induce positive adaptations in postmenopausal women (Nyberg et al., 2016; Lundberg Slingsby et al., 2017). The studies reporting clear beneficial effects, have involved women who are recently postmenopausal.
(i.e., <5 yrs. 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 a longer time to reach the same benefits, 2) higher intensity exercise may be more effective at compensating for the loss of estrogen 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 aging, 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 is 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.

**Estrogen Receptors, Estrogen-Related Receptor Alpha, Menopause, and Exercise Training**

Estrogen elicits its protective effects on the vasculature via two main estrogen receptor-mediated pathways in endothelial cells: 1) genomic regulation, involving the activation of estrogen 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 signaling...
cascades, e.g., increased phosphorylation of eNOS on Ser-1177 (Menazza & Murphy, 2016; Gliemann & Hellsten, 2019). Additionally, estrogen can post-translationally modify proteins via G protein-coupled estrogen receptor 1 (GPER) activation (Prossnitz & Barton, 2011). For example, GPER activation can turn on signaling cascades that promote eNOS activation (Fredette et al., 2018). The combined effects of estrogen and its receptors enhance both eNOS activity and NO bioavailability, which is critical for vasodilation 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 (Fürstermann & Münzel, 2006). This, in combination with the antioxidant properties of estrogen and its effect on upregulation of SOD2 and catalase, makes the role of estrogen in limiting oxidative stress and inflammation substantial (Ighodaro & Akinloye, 2018; Ribon-Demars et al., 2019). Lastly, estrogen 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, these estrogen-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 et al., 2012b) studies have suggested that estrogen 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. The estrogen receptor alpha (ERα) is purported to be
predominantly responsible for exerting the anti-inflammatory effects, while the estrogen receptor beta (ERβ) has been suggested to be related to a pro-inflammatory profile (Novella et al., 2012b). The loss of estrogen 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 estrogen by activating the orphan nuclear receptor estrogen-related receptor alpha (ERRα). In-vitro studies in skeletal muscle cell cultures have shown that muscle contraction activates ERE in the absence of estrogen, and that the effect is mitogen-activated protein kinase (MAPK)-dependent, indicating activation via ERRα (Wiik et al., 2009). This, combined with observations that ERα protein expression decreases after menopause (Novensà et al., 2011b; 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 signaling through the ERRα pathway may become more important. Notably, the ERRα pathway is coupled to the PGC1α pathway and promotes the production of several of the same key proteins related to vascular health as estrogen, e.g., eNOS, SOD2, as well as mitochondrial biogenesis (Perry et al., 2014; Craigie et al., 2016). Cumulatively, before menopause estrogenic effects can be achieved by both endogenous estrogen 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 and 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 35-74 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 (Staessen et al., 1997; Barton & Meyer, 2009). This accelerated rise is, at least in part, due to the hormonal changes occurring. Previous studies have suggested that the estrogen receptor GPER is the main receptor responsible for the potent eNOS-mediated vasodilatory effects of estrogen, 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 estrogen 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 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 et al., 2020a). 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 et al., 2020a).

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 (Nyberg et al., 2016; Hoier et al., 2021) and others high-intensity running, e.g., floorball and interval running (Nyberg et al., 2014; Gliemann et al., 2020b) and have found that these intensive exercise modalities produce beneficial reductions in arterial blood pressure (Fig. 3). Conversely, findings with

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low- to moderate-intensity training (e.g., walking and Nordic walking) are somewhat more
divergent, with some studies showing a lowering of blood pressure (di Blasio et al., 2012;
Cebula et al., 2020) and no effect in others (Pierce et al., 2011; Moreau et al., 2013).
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 dilation (FMD) is a non-invasive method that measures changes in artery
diameter with ultrasound Doppler in response to increased flow following reactive
hyperemia (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 hold 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 aging 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 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 estrogen 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 (>5 yrs. after menopause), changes in FMD appear difficult to achieve, regardless of type of exercise and site of measurement, but evidence also suggest that regular exercise with a combination of estrogen 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 imperative 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 preferable 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 (PGI2) analog epoprostenol, enables investigation of the ability of the endothelium in the smaller resistance arterioles to produce and secrete vasodilators and induce vasodilation 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 (R²=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 to decline with advanced aging.

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 (Nyberg et al., 2016; Gliemann et al., 2020b). The opposing findings regarding the effect of exercise on microvascular function in older women has 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 and 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, >5 years post menopause), the improvements in vascular function with a period of high-intensity floorball training have not been statistically significant (Gliemann et al., 2020b). 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 et al., 2020a). 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 (2020a), 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 shortly 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 die 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 (Periyah 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 aging, platelets become hyperreactive 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 estrogen is an important positive regulator of nitric oxide (NO) and PGI2 production, which are established inhibitors of platelet activation (Novella et al., 2012a). Consequently, it has been hypothesized that the loss of estrogen associated with menopause may promote an imbalance in platelet reactivity, favoring platelet hyperreactivity, 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.
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 hyperreactivity. 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 estrogen and may thus be an effective strategy to reduce platelet hyperreactivity in postmenopausal women. Notably, exercise training has been shown to increase circulating NO (Zaros et al., 2009; Esmail et al., 2011) and PGI₂ (Gliemann et al., 2020b) levels. Additionally, 3 months of high-intensity exercise training improved platelet PGI₂-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 viscoelastic 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 aging 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 estrogen with menopause may exacerbate these age-related hemostatic changes (Meilahn et al., 1992) and evidently, previous work using a different methodology has demonstrated that postmenopausal women form denser ex-vivo clots than premenopausal women (Piróg et al., 2016). Together, these findings may explain our observation of a higher \(d_f\) in postmenopausal women compared to young women.

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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 (Bonner et al., 2013; Akerstrom et al., 2014). 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 aging 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 stark sex-differences in the capillary-to-fiber ratio (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; Gries et al., 2018; Olsen et al., 2020; Gliemann et al., 2021; Perez-Gomez et al., 2021). For example, Gavin et al. (2014) demonstrated a ~20-25% increase in capillarization after 8 weeks of moderate-intensity training (heart rate equivalent to 65% of VO\textsubscript{2max}) 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 estrogen versus aging 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 (CD) 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, however data from our laboratory provides 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 is 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 aging may be reduced endothelial cell proliferation as well as the level of interstitial 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 estrogen, 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.

**Perspective**

In this review, we emphasize that regular physical activity is essential for healthy human aging. 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 is responsible for what is commonly considered vascular aging and accordingly, future studies should carefully consider the physical activity levels of participants when interpreting basal vascular data and the changes with aging 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, 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.

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 was approved by all authors.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

References


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**Graphical Abstract:** Compared to a physically inactive lifestyle, lifelong participation in physical activity protects against the development of vascular disease. However, aging 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 aging and menopause can be, at least partially, mitigated.
Figure 1: Linear correlations between VO$_{2\text{max}}$ and age, in sedentary women before (closed circles, n=76, unpublished data, Nyberg et al., 2016; Gliemann et al., 2020a; Hoier et al., 2021) and after an exercise intervention (8-12 weeks, open circles, n=52, Nyberg et al., 2016; Hoier et al., 2021) as well as a group of lifelong trained women (triangles, n=26, Gliemann et al., 2020a), both moderately and highly trained (moderately: 2-4 hours of low to moderate intensity exercise and 1 h of high-intensity exercise per week, highly: more than 4 hours of moderate- and high-intensity exercise per week). Significant relationship between VO$_{2\text{max}}$ 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 VO$_{2\text{max}}$ and age in the lifelong trained group ($R^2 = 0.004$ and $p = 0.764$). The data is 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.
Figure 2: Outlined differences in vascular health with active aging versus inactive aging in women. Active aging 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 aging women. Inactive aging 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 ($\text{O}_2^-$), accumulate and lead to lower nitric oxide (NO) bioavailability, which can impair endothelial function. Moreover, inactive aging is characterized by low capillary density and capillary-to-fiber (C:F) ratio. Importantly, initiating an exercise intervention can restore vascular health, although timing of the initiation of the interventions are of great importance.
**Figure 3**: Reductions in clinical systolic and diastolic blood pressure by exercise intervention in sedentary postmenopausal women (floorball, n=18, Spinning, n=27). Data adapted and combined from: Nyberg et al., 2014, 2016; Gunnarsson et al., 2020; Hoier et al., 2021.

**Figure 4**: Fractal dimension (d_f) analysis indicating increased strength and density of incipient blood clots in healthy postmenopausal women (n=27; age: 58 ± 5 years; 8 ± 5 years after menopause; VO_{2max}: 27.6 ± 5.6 mL·kg^{-1}·min^{-1}) compared to young, healthy women (n=8; age: 28 ± 2 years; VO_{2max}: 45.5 ± 2.4 mL·kg^{-1}·min^{-1}) (p = 0.005). All participants were fasted, avoided caffeine (24 hours), strenuous exercise (48 hours), and non-steroidal anti-inflammatory drugs (7 days). After resting in a supine position for 15 min, blood samples were drawn into vacutainers with no additive.
**Figure 5:** Linear correlations between capillary-to-fiber ratio (C:F ratio) and age, in sedentary women before (closed circles, n=61, (Olsen et al., 2020; Gliemann et al., 2021; 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 hours of low to moderate intensity exercise and 1 h of high-intensity exercise per week, highly: more than 4 hours of moderate- and high-intensity exercise per week). 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 $p = 0.03$). No linear relationships between C:F ratio and age in the lifelong trained group ($R^2 = 0.06$ and $p = 0.204$). Data is adapted and combined from previous published papers: Olsen et al., 2020; Gliemann et al., 2021; Perez-Gomez et al., 2021.
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