Stepping away from pharmaceutical therapies: Exercise and supplementation with fermented red clover extract as alternative strategies to promote vascular health in postmenopausal women

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In the middle of adversity there is great opportunity.

Albert Einstein

ABSTRACT

Cardiovascular disease is the leading cause of death worldwide. Both aging and menopause, associated with the cessation of endogenous estrogen production, are key factors that contribute to the development of cardiovascular disease in women. Over the last few decades, an interest in alternatives to pharmaceutical interventions for promoting and/or rescuing cardiovascular health in postmenopausal women has emerged, where both exercise and phytoestrogen supplementation have been deemed effective candidates. However, due to the paucity of intervention studies in postmenopausal women, knowledge gaps remain in these strategies that need to be elucidated in the context of vascular health. This dissertation aims to answer three main questions that will refine the scientific community's understanding of alternative interventions for vascular health in postmenopausal women: **(1)** Can exercise training work synergistically with *in-vitro* dual anti-platelet therapy to improve platelet function, as determined by basal platelet reactivity and prostacyclin sensitivity (*Chapter 4*)? **(2)** Does the timing of the initiation of exercise training after menopause affect the degree of vascular adaptations and thrombotic risk profile (*Chapters 5 and 6*)? **(3)** Can short-term supplementation with the novel phytoestrogen fermented red clover extract improve markers of vascular inflammation (*Chapter 7*)?

Together, the findings from this dissertation highlight that exercise and fermented red clover extract are effective alternative strategies to improving vascular health in postmenopausal women. Specifically, exercise training improves platelet function and

sensitivity and can work synergistically with *in-vitro* dual anti-platelet therapy (*Chapter 4*). In addition, short-term supplementation with fermented red clover extract improves the vascular inflammatory profile in recently postmenopausal women (*Chapter 7*). However, the timing of exercise training after menopause may influence the magnitude of thrombogenic adaptations, as recently postmenopausal women experience more robust thrombogenic benefits than women who are a greater number of years postmenopausal (i.e., late postmenopausal women) (*Chapter 5 and 6*).

Keywords: menopause, exercise, fermented red clover, vascular health, dual anti-platelet therapy

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1) Fischer, M.*, Jeppesen, J.S.*, Vigh-Larsen, J.F., Stöhr, E.J., Mohr, M., **Wickham, K.A.**, Gliemann, L., Bangsbo, J., Hellsten, Y., and Hostrup, M. 2023. Speed endurance training improves cardiac function but not intravascular blood volume of youth national team ice hockey players. Medicine & Science in Sports & Exercise (*submitted*).

2) Nørregaard, L.B.*, **Wickham, K.A.***, Ehlers, T., Rocha, M.P., Fischer, M., Lundberg Slingsby, M.H., Cheung, S.S., Evans, P.A., and Hellsten, Y. 2023. Exercise training induces thrombogenic benefits in recently but not late postmenopausal women. American Journal of Physiology – Heart and Circulatory Physiology (*submitted*). **(+)**

3) Hansen, C.V., Møller, S., **Wickham, K.A.**, Gliemann, L., and Hellsten, Y. 2022. Mitochondria derived reactive oxygen species lower NO bioavailability in microvascular endothelial cells: a study involving hypertension and MitoQ. Redox Biology (*submitted*).

4) **Wickham, K.A.** and Spriet, L.L. 2022. Food for Thought: Physiological Considerations for Nutritional Ergogenic Efficacy. *Scandinavian* Journal of Medicine and Science in Sports. https://doi.org/10.1111/sms.14307

5) **Wickham, K.A.**, Nørregaard, L.B., Lundberg Slingsby, M.H., Cheung, S.S., and Hellsten, Y. 2022. High-intensity exercise training improves basal platelet prostacyclin sensitivity and potentiates the response to dual anti-platelet therapy in postmenopausal women. Biomolecules. https://doi.org/10.3390/biom12101501 **(+)**

6) Tamariz-Ellemann, A.*, **Wickham, K.A.***, Nørregaard, L.B., Gliemann, L., and Hellsten, Y. 2022. The time is now: Regular exercise maintains vascular health in aging women. Journal of Physiology. https://doi.org/10.1113/JP282896. **(+)**

7) **Wickham, K.A.** and Cheung, S.S. 2022. Finger anthropometrics may not be a primary influence on the thermal responses to cooling and rewarming. Temperature. https://doi.org/10.1080/23328940.2022.2091901

8) **Wickham, K.A.***, Nørregaard, L.B.*, Oxfeldt, M., Cheung, S.S., Gliemann, L., Hansen, M., and Hellsten, Y. 2022. Short-term Supplementation with Fermented Red Clover Extract Reduces Vascular Inflammation in Early Post-menopausal Women. Frontiers in Cardiovascular Medicine. https://doi.org/10.3389/fcvm.2022.826959 **(+)**

9) Hansen, C., Møller, S., Ehlers, T., **Wickham, K.A.**, Bangsbo, J., Gliemann, L., and Hellsten, Y. 2022. Redox balance in human skeletal muscle-derived endothelial cells - Effect of exercise training. Free Radical Biology and Medicine. 10.1016/j.freeradbiomed.2021.12.265

10) **Wickham, K.A.**, McCarthy, D.G., Spriet, L.L., and Cheung, S.S. 2021. Sex differences in the physiological responses to exercise-induced dehydration: Consequences and mechanisms? Journal of Applied Physiology. 10.1152/japplphysiol.00266.2021.

11) **Wickham, K.A.**, Steele, S.W., and Cheung, S.S. 2021. Effects of acute dietary nitrate supplementation on cold-induced vasodilation in healthy males. European Journal of Applied Physiology. 10.1007/s00421-021-04621-8.

12) **Wickham, K.A.**, Wallace, P.J., and Cheung, S.S. 2021. Sex differences in the physiological adaptations to heat acclimation: a state-of-the-art review. European Journal of Applied Physiology. 10.1007/s00421-020-04550-y.

13) **Wickham, K.A.** 2020. Commentary: Investigators should/should not control for menstrual cycle phase when performing studies of vascular control that include women. Journal of Applied Physiology. 10.1152/japplphysiol.00809.2020.

14) Jannas-Vela, S., Klingel, S.L., Cervone, D.T., **Wickham, K.A.**, Heigenhauser, G., Mutch, D.M., Holloway, G., and Spriet, L.L. 2020. Resting metabolic rate and skeletal muscle SERCA and Na+/K+ ATPase activities are not affected by fish oil supplementation in healthy older adults. Physiological Reports. 10.14814/phy2.14408.

15) **Wickham, K.A.**, Nyakayiru, J., McCarthy, D.G., and Cervone, D.T. 2019. Skeletal muscle nitrate storage – the missing piece of the nitrate supplementation puzzle. Journal of Physiology. 10.1113/jp278785.

16) Cervone, D.T., Hucik, B., and **Wickham, K.A.** 2019. Can ketone esters support an appetite to perform and recover? Journal of Physiology. 10.1113/jp278462.

17) **Wickham, K.A.** and Spriet, L.L. 2019. No longer beeting around the bush: A review of potential sex differences with dietary nitrate supplementation. Applied Physiology, Nutrition, and Metabolism. 10.1139/apnm-2019-0063.

18) McCarthy, D.G., **Wickham, K.A.**, Vermeulen, T., Nyman, D.L., Ferth, S., Pereira, J.M., Larson, D.J., Burr, J.F., and Spriet, L.L. 2019. Impairment of thermoregulation and performance via mild dehydration in ice hockey goaltenders. International Journal of Sports Physiology and Performance. 10.1123/ijspp.2019-0464.

19) **Wickham, K.A.**, McCarthy, D.G., Cervone, D.T., Verdijk, L.B., van Loon, L.J.C., and Spriet, L.L. 2019. No effect of beetroot juice supplementation on exercise economy and performance in recreationally active females despite increased torque production. Physiological Reports. 10.14814/phy2.13982.

20) **Wickham, K.A.** and Spriet, L.L. 2018. Administration of Caffeine in Alternate Forms. Sports Medicine. 10.1007/s40279-017-0848-2.

LIST OF ABBREVIATIONS

$[Ca^{2+}]_i$	Intracellular Calcium
6-Keto $PGF_{1\alpha}$	6-Keto Prostaglandin $F_{1\alpha}$
AA	Arachidonic Acid
ACh	Acetylcholine
ADP	Adenosine 5’-Diphosphate
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BCA	Bicinchoninic Acid
BH_4	Tetrahydro-L-Biopterin
BMI	Body Mass Index
Ca^{2+}	Calcium
C:F Ratio	Capillary to Fiber Ratio
cAMP	Cyclic Adenosine Monophosphate
CD	Capillary Density
CD31	Cluster of Differentiation 31
COX1	Cyclooxygenase-1

COVID-19	Novel Coronavirus
CRP	C-Reactive Protein
DAPT	Dual Anti-Platelet Therapy
d_f	Fractal Dimension
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
DXA	Dual Energy X-ray Absorptiometry
ECG	Electrocardiogram
ECL	Luminata Forte
ELISA	Enzyme-Linked Immunosorbent Assay
EDTA	Ethylenediaminetetraacetic Acid
eNOS	Endothelial Nitric Oxide Synthase
ERα	Estrogen Receptor Alpha
ERβ	Estrogen Receptor Beta
ERR	Estrogen-Related Receptors
ERRα	Estrogen-Related Receptor Alpha

FEMTO	SuperSignal™ West Femto Maximum Sensitivity Substrate
FLK1	Fetal Liver Kinase 1
FMD	Flow-Mediated Dilation
GP	Gel Point
GPER	G Protein-Coupled Estrogen Receptor 1
GPVI	Glycoprotein VI
HEPES	4-(2-Hydroxyethyl)-1-Piperazineethanesulfonic Acid
HR	Heart Rate
HR_{max}	Maximal Heart Rate
HRT	Hormone Replacement Therapy
HRP	Horseradish Peroxidase
ICAM-1	Intracellular Adhesion Molecule 1
IPAQ	International Physical Activity Questionnaire
KCl	Potassium Chloride
LPW	Late Postmenopausal Women
$MgCl_2$	Magnesium Chloride
mmHg	Millimeters of Mercury

NaCl	Sodium Chloride
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NaF	Sodium Fluoride
$NaHCO_3$	Sodium Bicarbonate
Na_3PO_4	Trisodium Phosphate
NO	Nitric Oxide
NP-40	Nonyl Phenoxypolyethoxylethanol
O_2	Oxygen
OX-PHOS	Oxidative Phosphorylation
PAI-1	Plasminogen Activator Inhibitor 1
PAR	Protease-Activated Receptors
PBS	Phosphate-Buffered Saline
PCG1α	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha
PGI_2	Prostacyclin
PGI_2S	Prostacyclin Synthase
PLA	Placebo
PMSF	Phenylmethylsulfonyl Fluoride

PPP	Platelet-Poor Plasma
PRP	Platelet-Rich Plasma
PVDF	Polyvinylidene Difluoride
RBC	Red Blood Cells
RC	Fermented Red Clover Extract
ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute
RPW	Recently Postmenopausal Women
SAA	Serum Amyloid A
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SOD2	Superoxide Dismutase 2
TBST	Tris-Buffered Saline Tween
TF	Tissue Factor
TGX	Tris-Glycine eXtended
TPA	Plasminogen Activator
TRAP-6	Thrombin Receptor Activator Peptide 6

TXA_2	Thromboxane A_2
TXA_2R	Thromboxane A_2 Receptor
TXA_2S	Thromboxane A_2 Synthase
TXB_2	Thromboxane B_2
VCAM-1	Vascular Cell Adhesion Molecule 1
VEGFR2	Vascular Endothelial Growth Factor Receptor 2
$\dot{V}O_{2max}$	Maximal Oxygen Uptake
vWF	Von Willebrand Factor
W	Watts
W_{max}	Watt Maximum
WBC	White Blood Cells

CHAPTER 1 GENERAL INTRODUCTION

Two hundred and fifteen million liters. This is the average volume of blood pumped and distributed by the heart and vascular system over a lifetime (Pomeroy et al., 2020). This feat is vital for the delivery of oxygen and nutrients to all cells of the body (Pomeroy et al., 2020), and optimal function of this highly coordinated system is crucial for maximizing an individual's health- and lifespan. Over the last 30 years, the global prevalence of cardiovascular disease has risen dramatically. In 2019, an estimated 17.9 million people died from cardiovascular disease worldwide, accounting for one-third of all deaths (Roth et al., 2020). Notably, compared to men, the absolute number of women living with and dying from cardiovascular disease is higher (Roger et al., 2011). Both aging and menopause, associated with the cessation of endogenous estrogen production, are key factors that contribute to the development of cardiovascular disease in women (Parker et al., 2010).

Recently, there has been a movement away from relying solely on pharmacological interventions for disease prevention and treatment (Thompson et al., 2020). This movement is at least partially driven by a growing body of evidence highlighting adverse side effects, only short-term benefits, and the potential for drug-drug interactions that may limit the effectiveness of the treatment. Importantly, increasing age and sex are known factors that influence the likelihood of adverse drug reactions. Specifically, women are at a 1.5- to 1.7-fold greater risk of developing adverse drug reactions compared to males, which may be related to pharmaceutical drug use, pharmacokinetic differences,

and higher absolute doses (Rademaker, 2001). Accordingly, it has become imperative to find safe, effective, and long-lasting strategies to promote cardiovascular health in postmenopausal women.

The purpose of this dissertation is to explore alternative strategies for improving vascular health in postmenopausal women, which may help promote a reduced reliance on drug-related interventions. This dissertation will specifically focus on interventions utilizing regular physical activity or phytoestrogen supplementation. The research program consists of three separate research projects described in *Chapters 4, 6, and 7* as well as a review paper described in *Chapter 5*. The aims of these projects are summarized in *Chapter 3*. Briefly, *Chapter 4* examines the impact of exercise training on platelet reactivity and drug-responsiveness to dual anti-platelet therapy (DAPT). *Chapter 5* provides a commentary on and analysis of the impact of time/years after menopause on vascular health and adaptations to regular physical activity. *Chapter 6* investigates the impact of time/years after menopause of vascular adaptations to exercise training. Lastly, *Chapter 7* tests the impact of short-term fermented red clover extract supplementation on blood and skeletal muscle markers of vascular health. Together, the findings from this dissertation will use a combination of applied and mechanistic investigations to advance the scientific community's understanding of the role of exercise training and fermented red clover supplementation on vascular health in postmenopausal women. These findings may hold societal relevance regarding recommendations for the promotion and initiation of exercise training in postmenopausal women.

1.1 References

Parker, B.A., Kalasky, M.J., Proctor, D.N., 2010. Evidence for sex differences in cardiovascular aging and adaptive responses to physical activity. Eur J Appl Physiol 110, 235–246. https://doi.org/10.1007/s00421-010-1506-7

Pomeroy, J.E., Helfer, A., Bursac, N., 2020. Biomaterializing the Promise of Cardiac Tissue Engineering. Biotechnol Adv 42, 107353. https://doi.org/10.1016/j.biotechadv.2019.02.009

Rademaker, M., 2001. Do women have more adverse drug reactions? Am J Clin Dermatol 2, 349–351. https://doi.org/10.2165/00128071-200102060-00001

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Adams, R.J., Berry, J.D., Brown, T.M., Carnethon, M.R., Dai, S., de Simone, G., Ford, E.S., Fox, C.S., Fullerton, H.J., Gillespie, C., Greenlund, K.J., Hailpern, S.M., Heit, J.A., Ho, P.M., Howard, V.J., Kissela, B.M., Kittner, S.J., Lackland, D.T., Lichtman, J.H., Lisabeth, L.D., Makuc, D.M., Marcus, G.M., Marelli, A., Matchar, D.B., McDermott, M.M., Meigs, J.B., Moy, C.S., Mozaffarian, D., Mussolino, M.E., Nichol, G., Paynter, N.P., Rosamond, W.D., Sorlie, P.D., Stafford, R.S., Turan, T.N., Turner, M.B., Wong, N.D., Wylie-Rosett, J., 2011. Heart Disease and Stroke Statistics—2011 Update. Circulation 123, e18–e209. https://doi.org/10.1161/CIR.0b013e3182009701

Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noël C., Beaton, A.Z., Benjamin, E.J., Benziger, C.P., Bonny, Aimé, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J., Catapano, A.L., Chugh, S.S., Cooper, L.T., Coresh, J., Criqui, M., DeCleene, N., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Solà, J., Fowkes, G., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N., Koroshetz, W., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Temesgen, A.M., Mokdad, A., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Moraes de Oliveira, G., Otto, C., Owolabi, M., Pratt, M., Rajagopalan, S., Reitsma, M., Ribeiro, A.L.P., Rigotti, N., Rodgers, A., Sable, C., Shakil, S., Sliwa-Hahnle, K., Stark, B., Sundström, J., Timpel, P., Tleyjeh, I.M., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L., Murray, C., Fuster, V., Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noel C., Beaton, A., Benjamin, E.J., Benziger, C.P., Bonny, Aime, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J.R., Catapano, A.L., Chugh, S., Cooper, L.T., Coresh, J., Criqui, M.H., DeCleene, N.K., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Sola, J., Fowkes, F.G.R., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N.J., Koroshetz, W.J., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Misganaw, A.T., Mokdad, A.H., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Oliveira, G.M.M., Otto, C.M., Owolabi, M.O., Pratt, M., Rajagopalan, S., Reitsma, M.B., Ribeiro, A.L.P., Rigotti, N.A., Rodgers, A., Sable, C.A., Shakil, S.S., Sliwa, K., Stark, B.A., Sundström, J., Timpel, P., Tleyjeh, I.I., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L.J., Abbasi-Kangevari, M., Abdi, A., Abedi, A., Aboyans, V., Abrha, W.A., Abu-

Gharbieh, E., Abushouk, A.I., Acharya, D., Adair, T., Adebayo, O.M., Ademi, Z., Advani, S.M., Afshari, K., Afshin, A., Agarwal, G., Agasthi, P., Ahmad, S., Ahmadi, S., Ahmed, M.B., Aji, B., Akalu, Y., Akande-Sholabi, W., Aklilu, A., Akunna, C.J., Alahdab, F., Al-Eyadhy, A., Alhabib, K.F., Alif, S.M., Alipour, V., Aljunid, S.M., Alla, F., Almasi-Hashiani, A., Almustanyir, S., Al-Raddadi, R.M., Amegah, A.K., Amini, S., Aminorroaya, A., Amu, H., Amugsi, D.A., Ancuceanu, R., Anderlini, D., Andrei, T., Andrei, C.L., Ansari-Moghaddam, A., Anteneh, Z.A., Antonazzo, I.C., Antony, B., Anwer, R., Appiah, L.T., Arabloo, J., Ärnlöv, J., Artanti, K.D., Ataro, Z., Ausloos, M., Avila-Burgos, L., Awan, A.T., Awoke, M.A., Ayele, H.T., Ayza, M.A., Azari, S., B, D.B., Baheiraei, N., Baig, A.A., Bakhtiari, A., Banach, M., Banik, P.C., Baptista, E.A., Barboza, M.A., Barua, L., Basu, S., Bedi, N., Béjot, Y., Bennett, D.A., Bensenor, I.M., Berman, A.E., Bezabih, Y.M., Bhagavathula, A.S., Bhaskar, S., Bhattacharyya, K., Bijani, A., Bikbov, B., Birhanu, M.M., Boloor, A., Brant, L.C., Brenner, H., Briko, N.I., Butt, Z.A., Caetano dos Santos, F.L., Cahill, L.E., Cahuana-Hurtado, L., Cámera, L.A., Campos-Nonato, I.R., Cantu-Brito, C., Car, J., Carrero, J.J., Carvalho, F., Castañeda-Orjuela, C.A., Catalá-López, F., Cerin, E., Charan, J., Chattu, V.K., Chen, S., Chin, K.L., Choi, J.-Y.J., Chu, D.-T., Chung, S.-C., Cirillo, M., Coffey, S., Conti, S., Costa, V.M., Cundiff, D.K., Dadras, O., Dagnew, B., Dai, X., Damasceno, A.A.M., Dandona, L., Dandona, R., Davletov, K., De la Cruz-Góngora, V., De la Hoz, F.P., De Neve, J.-W., Denova-Gutiérrez, E., Derbew Molla, M., Derseh, B.T., Desai, R., Deuschl, G., Dharmaratne, S.D., Dhimal, M., Dhungana, R.R., Dianatinasab, M., Diaz, D., Djalalinia, S., Dokova, K., Douiri, A., Duncan, B.B., Duraes, A.R., Eagan, A.W., Ebtehaj, S., Eftekhari, A., Eftekharzadeh, S., Ekholuenetale, M., El Nahas, N., Elgendy, I.Y., Elhadi, M., El-Jaafary, S.I., Esteghamati, S., Etisso, A.E., Eyawo, O., Fadhil, I., Faraon, E.J.A., Faris, P.S., Farwati, M., Farzadfar, F., Fernandes, E., Fernandez Prendes, C., Ferrara, P., Filip, I., Fischer, F., Flood, D., Fukumoto, T., Gad, M.M., Gaidhane, S., Ganji, M., Garg, J., Gebre, A.K., Gebregiorgis, B.G., Gebregzabiher, K.Z., Gebremeskel, G.G., Getacher, L., Obsa, A.G., Ghajar, A., Ghashghaee, A., Ghith, N., Giampaoli, S., Gilani, S.A., Gill, P.S., Gillum, R.F., Glushkova, E.V., Gnedovskaya, E.V., Golechha, M., Gonfa, K.B., Goudarzian, A.H., Goulart, A.C., Guadamuz, J.S., Guha, A., Guo, Y., Gupta, R., Hachinski, V., Hafezi-Nejad, N., Haile, T.G., Hamadeh, R.R., Hamidi, S., Hankey, G.J., Hargono, A., Hartono, R.K., Hashemian, M., Hashi, A., Hassan, S., Hassen, H.Y., Havmoeller, R.J., Hay, S.I., Hayat, K., Heidari, G., Herteliu, C., Holla, R., Hosseini, M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Househ, M., Huang, J., Humayun, A., Iavicoli, I., Ibeneme, C.U., Ibitoye, S.E., Ilesanmi, O.S., Ilic, I.M., Ilic, M.D., Iqbal, U., Irvani, S.S.N., Islam, S.M.S., Islam, R.M., Iso, H., Iwagami, M., Jain, V., Javaheri, T., Jayapal, S.K., Jayaram, S., Jayawardena, R., Jeemon, P., Jha, R.P., Jonas, J.B., Jonnagaddala, J., Joukar, F., Jozwiak, J.J., Jürisson, M., Kabir, A., Kahlon, T., Kalani, R., Kalhor, R., Kamath, A., Kamel, I., Kandel, H., Kandel, A., Karch, A., Kasa, A.S., Katoto, P.D.M.C., Kayode, G.A., Khader, Y.S., Khammarnia, M., Khan, M.S., Khan, M.N., Khan, M.,

Khan, E.A., Khatab, K., Kibria, G.M.A., Kim, Y.J., Kim, G.R., Kimokoti, R.W., Kisa, S., Kisa, A., Kivimäki, M., Kolte, D., Koolivand, A., Korshunov, V.A., Koulmane Laxminarayana, S.L., Koyanagi, A., Krishan, K., Krishnamoorthy, V., Kuate Defo, B., Kucuk Bicer, B., Kulkarni, V., Kumar, G.A., Kumar, N., Kurmi, O.P., Kusuma, D., Kwan, G.F., La Vecchia, C., Lacey, B., Lallukka, T., Lan, Q., Lasrado, S., Lassi, Z.S., Lauriola, P., Lawrence, W.R., Laxmaiah, A., LeGrand, K.E., Li, M.-C., Li, B., Li, S., Lim, S.S., Lim, L.-L., Lin, H., Lin, Z., Lin, R.-T., Liu, X., Lopez, A.D., Lorkowski, S., Lotufo, P.A., Lugo, A., M, N.K., Madotto, F., Mahmoudi, M., Majeed, A., Malekzadeh, R., Malik, A.A., Mamun, A.A., Manafi, N., Mansournia, M.A., Mantovani, L.G., Martini, S., Mathur, M.R., Mazzaglia, G., Mehata, S., Mehndiratta, M.M., Meier, T., Menezes, R.G., Meretoja, A., Mestrovic, T., Miazgowski, B., Miazgowski, T., Michalek, I.M., Miller, T.R., Mirrakhimov, E.M., Mirzaei, H., Moazen, B., Moghadaszadeh, M., Mohammad, Y., Mohammad, D.K., Mohammed, S., Mohammed, M.A., Mokhayeri, Y., Molokhia, M., Montasir, A.A., Moradi, G., Moradzadeh, R., Moraga, P., Morawska, L., Moreno Velásquez, I., Morze, J., Mubarik, S., Muruet, W., Musa, K.I., Nagarajan, A.J., Nalini, M., Nangia, V., Naqvi, A.A., Narasimha Swamy, S., Nascimento, B.R., Nayak, V.C., Nazari, J., Nazarzadeh, M., Negoi, R.I., Neupane Kandel, S., Nguyen, H.L.T., Nixon, M.R., Norrving, B., Noubiap, J.J., Nouthe, B.E., Nowak, C., Odukoya, O.O., Ogbo, F.A., Olagunju, A.T., Orru, H., Ortiz, A., Ostroff, S.M., Padubidri, J.R., Palladino, R., Pana, A., Panda-Jonas, S., Parekh, U., Park, E.-C., Parvizi, M., Pashazadeh Kan, F., Patel, U.K., Pathak, M., Paudel, R., Pepito, V.C.F., Perianayagam, A., Perico, N., Pham, H.Q., Pilgrim, T., Piradov, M.A., Pishgar, F., Podder, V., Polibin, R.V., Pourshams, A., Pribadi, D.R.A., Rabiee, N., Rabiee, M., Radfar, A., Rafiei, A., Rahim, F., Rahimi-Movaghar, V., Ur Rahman, M.H., Rahman, M.A., Rahmani, A.M., Rakovac, I., Ram, P., Ramalingam, S., Rana, J., Ranasinghe, P., Rao, S.J., Rathi, P., Rawal, L., Rawasia, W.F., Rawassizadeh, R., Remuzzi, G., Renzaho, A.M.N., Rezapour, A., Riahi, S.M., Roberts-Thomson, R.L., Roever, L., Rohloff, P., Romoli, M., Roshandel, G., Rwegerera, G.M., Saadatagah, S., Saber-Ayad, M.M., Sabour, S., Sacco, S., Sadeghi, M., Saeedi Moghaddam, S., Safari, S., Sahebkar, A., Salehi, S., Salimzadeh, H., Samaei, M., Samy, A.M., Santos, I.S., Santric-Milicevic, M.M., Sarrafzadegan, N., Sarveazad, A., Sathish, T., Sawhney, M., Saylan, M., Schmidt, M.I., Schutte, A.E., Senthilkumaran, S., Sepanlou, S.G., Sha, F., Shahabi, S., Shahid, I., Shaikh, M.A., Shamali, M., Shamsizadeh, M., Shawon, M.S.R., Sheikh, A., Shigematsu, M., Shin, M.-J., Shin, J.I., Shiri, R., Shiue, I., Shuval, K., Siabani, S., Siddiqi, T.J., Silva, D.A.S., Singh, J.A., Mtech, A.S., Skryabin, V.Y., Skryabina, A.A., Soheili, A., Spurlock, E.E., Stockfelt, L., Stortecky, S., Stranges, S., Suliankatchi Abdulkader, R., Tadbiri, H., Tadesse, E.G., Tadesse, D.B., Tajdini, M., Tariqujjaman, M., Teklehaimanot, B.F., Temsah, M.-H., Tesema, A.K., Thakur, B., Thankappan, K.R., Thapar, R., Thrift, A.G., Timalsina, B., Tonelli, M., Touvier, M., Tovani-Palone, M.R., Tripathi, A., Tripathy, J.P., Truelsen, T.C., Tsegay, G.M., Tsegaye, G.W., Tsilimparis, N., Tusa, B.S.,

Tyrovolas, S., Umapathi, K.K., Unim, B., Unnikrishnan, B., Usman, M.S., Vaduganathan, M., Valdez, P.R., Vasankari, T.J., Velazquez, D.Z., Venketasubramanian, N., Vu, G.T., Vujcic, I.S., Waheed, Y., Wang, Y., Wang, F., Wei, J., Weintraub, R.G., Weldemariam, A.H., Westerman, R., Winkler, A.S., Wiysonge, C.S., Wolfe, C.D.A., Wubishet, B.L., Xu, G., Yadollahpour, A., Yamagishi, K., Yan, L.L., Yandrapalli, S., Yano, Y., Yatsuya, H., Yeheyis, T.Y., Yeshaw, Y., Yilgwan, C.S., Yonemoto, N., Yu, C., Yusefzadeh, H., Zachariah, G., Zaman, S.B., Zaman, M.S., Zamanian, M., Zand, R., Zandifar, A., Zarghi, A., Zastrozhin, M.S., Zastrozhina, A., Zhang, Z.-J., Zhang, Y., Zhang, W., Zhong, C., Zou, Z., Zuniga, Y.M.H., Murray, C.J.L., Fuster, V., 2020. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology 76, 2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010

Thompson, W.R., Sallis, R., Joy, E., Jaworski, C.A., Stuhr, R.M., Trilk, J.L., 2020. Exercise Is Medicine. American Journal of Lifestyle Medicine 14, 511–523. https://doi.org/10.1177/1559827620912192

CHAPTER 2 REVIEW OF THE LITERATURE

The prevalence of cardiovascular disease is significantly lower in young women compared to age-matched men (Bittner, 2009). This is often attributed to the cardioprotective effects of estrogens (El Khoudary et al., 2020). Accordingly, after the menopausal transition, marked by the cessation of endogenous estrogen production, the incidence of cardiovascular disease in women matches or surpasses that of age-matched men (Bittner, 2009; El Khoudary et al., 2020). This finding has initiated an ongoing pursuit of therapies that can attenuate the menopause-associated declines in cardiovascular health in women. Over the last few decades, the administration of exogenous hormones, termed estrogen and progestogen hormone replacement therapy (HRT), has been at the forefront of this venture (Grady et al., 1992; Hackley and Rousseau, 2004). However, HRT has recently been associated with the development of certain cancers and other adverse health outcomes (Nelson et al., 2002; Rossouw et al., 2002), sparking an urgent call for novel, safe, and effective alternatives for postmenopausal women. Thus, the focus of this literature review is to provide a brief overview of vascular biology as well as summarize the current knowledge on vascular health in postmenopausal women and how alternative interventions, such as exercise training and phytoestrogen supplementation, can benefit vascular health in this cohort.

2.1 Cardiovascular System

The cardiovascular system consists of the heart, the vasculature, and the blood, which are critical for the delivery of oxygen and nutrients as well as the removal of waste products

from all tissues (Pomeroy et al., 2020). The vasculature is comprised of arteries, arterioles, capillaries, veins, and venules, and is a hierarchical network based on vessel diameter that precisely regulates blood flow to tissues and organs as well as the return of blood to the heart (Yuan and Rigor, 2010). The blood is comprised of plasma, red blood cells, white blood cells, and platelets (Garraud and Tissot, 2018). Approximately 55% of blood is plasma, which is predominantly composed of water with proteins, ions, nutrients, and waste products. Red blood cells are critical for delivering oxygen and removing carbon dioxide from tissues, whereas white blood cells participate in immune function, and platelets play a critical role in blood clotting (Garraud and Tissot, 2018). This dissertation will focus on the vasculature and the blood, with a particular emphasis on platelets.

2.1.1 Macrovasculature

The macrovasculature is comprised of large elastic arteries (i.e., conduit arteries) and veins. The large conduit arteries are important for quickly delivering blood to tissues, whereas the veins are critical for returning blood to the heart (Yuan and Rigor, 2010). Due to their elastic nature, which allows for large changes in vessel diameter, conduit arteries play a minor role in the regulation of vascular resistance and blood flow under normal physiological conditions (Levick, 1991).

2.1.2 Microvasculature

The microvasculature is comprised of small vessels including arterioles, capillaries, and venules (Yuan and Rigor, 2010). These microvessels form a comprehensive network that regulates local blood perfusion and facilitates blood-tissue exchange (Levick, 1991). The

anatomical structure of resistance arterioles, consisting of a single layer of endothelial cells enveloped by a thick layer of smooth muscle cells, allows for strict control of local blood flow (Levick, 1991). Specifically, contraction and relaxation of the smooth muscle cells allows for rapid and dynamic control of vessel diameter, and subsequently blood flow (Levick, 1991).

2.1.3 Capillary Network

Capillaries are the smallest blood vessels and their primary role is to facilitate the delivery of oxygen and nutrients as well as the removal of waste products between the blood and tissues (Yuan and Rigor, 2010). Capillaries are thin-walled vessels composed of a single layer of endothelial cells surrounded by a basement membrane (Levick, 1991); these characteristics make them ideal candidates to optimally facilitate this exchange.

Angiogenesis is a process that increases the number of capillaries in response to physiological stressors, such as exercise training and tumour growth (Wagner, 2011). Angiogenesis can increase the efficiency of oxygen and nutrient delivery as well as waste removal from a target tissue (Wagner, 2011) and is a fundamental process in health and disease. Notably, vascular endothelial growth factor (VEGF) is a potent angiogenic factor that facilitates capillary growth.

2.1.4 Vascular Endothelium

Endothelial cells line the luminal surface of all blood vessels and play key roles in the regulation and communication of the cardiovascular system (Levick, 1991). Endothelial cells produce and release a multitude of compounds that can communicate with the

surrounding smooth muscle and platelets. For example, nitric oxide (NO) and prostacyclin released from endothelial cells interact with the surrounding smooth muscle cells to potently promote vasodilation (Hamilos et al., 2018), while also playing roles in the inhibition of platelet activation in the blood (Wang et al., 1998). Conversely, endothelial cells also release endothelin-1 and inflammatory markers (e.g., tumour necrosis factor α), which promote vasoconstriction of the surrounding smooth muscle cells (Westby et al., 2011) and platelet activation in the blood (Pignatelli et al., 2005), respectively.

NO is ubiquitously expressed, and its production is facilitated by isoforms of nitric oxide synthase (NOS) (Jones and Bolli, 2006). Specifically, endothelial NO is produced by endothelial nitric oxide synthase (eNOS) (Förstermann and Münzel, 2006), which is activated by muscarinic, purinergic, and mechanoreceptors (Fleming, 2010). Various stimuli (e.g., acetylcholine (ACh), adenosine triphosphate (ATP), adenosine, and stretch or shear stress) activate these receptors, which increases intracellular calcium $[Ca^{2+}]_i$ and results in calmodulin binding to eNOS (Fleming, 2010). This triggers the phosphorylation of eNOS on several activation sites (e.g., serine-1177 and serine-635) (Fleming, 2010). Activated eNOS forms dimers, which bind to the coenzyme tetrahydro-L-biopterin (BH_4). This allows eNOS to convert L-arginine, oxygen, and nicotinamide adenine dinucleotide phosphate (NADPH) into NO and L-citrulline (Fleming, 2010) (Figure 2-1).

Akin to NO, prostacyclin is formed in response to elevated $[Ca^{2+}]_i$. This leads to the liberation of arachidonic acid and activation of prostacyclin synthase (Whorton et al., 1984), an enzyme critical for prostacyclin formation. Specifically, prostacyclin is synthesized by a two-step conversion (Whorton et al., 1984). First, arachidonic acid is

converted to prostaglandin H_2 via the enzyme cyclooxygenase-1. Second, prostaglandin H_2 is converted to prostacyclin via the enzyme prostacyclin synthase (Whorton et al., 1984) (Figure 2-1).

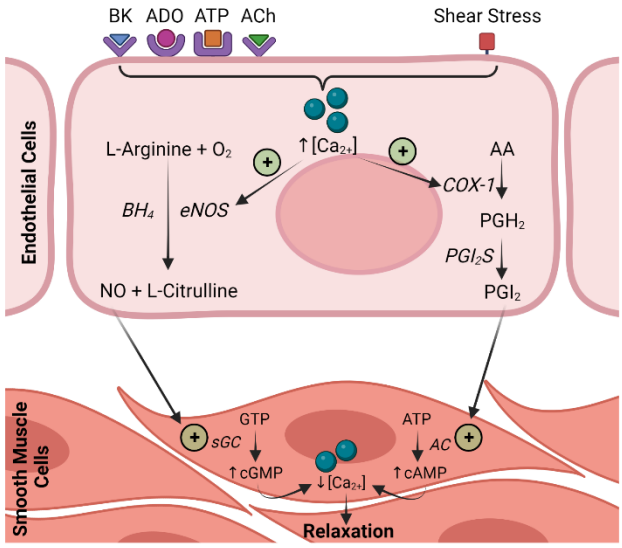

Figure 2-1: Endothelial cell production of nitric oxide (NO) and prostacyclin (PGI_2).

The interaction of ligands with receptors for bradykinin (BK), adenosine (ADO), adenosine triphosphate (ATP), acetylcholine (ACh), and by shear stress to increase intracellular calcium ($[Ca^{2+}]_i$). This activates endothelial nitric oxide synthase (eNOS) to produce nitric oxide (NO) and cyclooxygenase-1 (COX1) to produce the downstream target prostacyclin (PGI_2). In vascular smooth muscle cells, NO stimulates soluble guanylyl cyclase (sGC) to increase cycling guanosine monophosphate (cGMP) and PGI_2 stimulates adenylyl cyclase (AC) to increase cyclic adenosine monophosphate (cAMP). Both cGMP and cAMP $[Ca^{2+}]_i$ in the smooth muscle cells, resulting in relaxation. O_2; oxygen, BH_4; tetrahydro-L-biopterin, AA; arachidonic acid, PGH_2; prostaglandin H_2, PGI_2S; prostacyclin synthase (Wickham, *unpublished*).

2.1.5 Platelets

Platelets, otherwise known as thrombocytes, are small anucleate blood cells (0.3 µm by 0.5 µm) derived from megakaryocytes in the bone marrow (Batty and Smith, 2010). Due to their shape and small size, platelets are pushed to the edges of blood vessels, allowing them to have close interactions with endothelial cells (Batty and Smith, 2010). This is crucial, as platelets are primarily involved in facilitating blood clot formation at sites of vascular injury, which occur on the basement membrane of endothelial cells (Periayah et al., 2017). Platelets have several membrane-bound receptors that are critical for platelet

activation and the clotting process (Periayah et al., 2017). Moreover, platelets house various storage granules that are important for regulating platelet function and blood clot formation. The α-granules contain coagulation factors (fibrinogen, Factor V, Factor VIII, von Willebrand factor (vWF)) and membrane proteins (αIIbβ3 and P-selectin) critical for blood clotting, as well as fibrinolytic inhibitors that prevent clot dissolution (plasminogen, PAI-1, and α2-antiplasmin) (White, 1998). Conversely, the dense granules store Ca^{2+} ions and small organic molecules (such as adenosine 5'-diphosphate (ADP) and serotonin) (Hanby et al., 2017) (Figure 2-2). A single platelet contains ~50 to 80 α-granules (Yun et al., 2016) and ~3 to 8 dense granules (White, 1998). A normal platelet count ranges from 150 to 400 x 10^9 platelets·L^{-1} (Batty and Smith, 2010). The role of platelets in primary hemostasis will be discussed in detail in the next section.

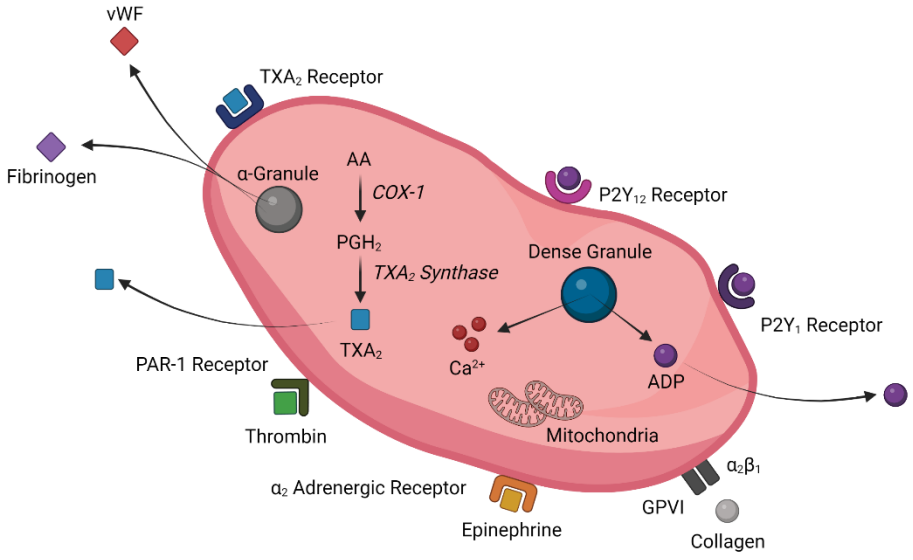

Figure 2-2: Basic schematic of a platelet.

The schematic includes the major storage molecules (α-granules and dense granules) as well as platelet membrane receptors and agonists. vWF; von Willebrand factor, AA; arachidonic acid, COX-1; cyclooxygenase-1, PGH_2; prostaglandin H_2, TXA_2; thromboxane A_2, ADP; adenosine 5'-diphosphate; Ca^{2+}; calcium, GPVI; glycoprotein VI (Wickham, *unpublished*).

2.1.6 Cardiovascular Disease

Cardiovascular disease is divided into four major categories: coronary artery disease, cerebrovascular disease, peripheral artery disease, and aortic atherosclerosis (Olvera Lopez et al., 2022). Atherosclerosis, a chronic lipid-driven inflammatory disease of the arteries, is a common denominator in the development of all major categories of cardiovascular disease (Olvera Lopez et al., 2022). Initially, atherosclerosis is characterized by a chronic inflammatory state that impairs blood vessel function. Over time this progresses to advanced atherosclerosis, which results in structural changes to the vasculature, such as stiffening and narrowing of the arteries (Alfarisi et al., 2020). This reduces and potentially occludes blood flow through the artery, which can trigger cardiovascular events, such as myocardial infarctions or stroke (Alfarisi et al., 2020). Critically, 90% of the risk of having a first myocardial infarction can be attributed to nine modifiable, and deeply interconnected, risk factors: smoking, dyslipidemia, hypertension, diabetes, abdominal obesity, psychosocial factors, poor fruit and vegetable consumption, regular alcohol consumption, and physical inactivity (Yusuf et al., 2004).

2.2 Hemostasis

When a blood vessel is injured, hemostasis occurs to form a blood clot and ultimately stop bleeding at the site of injury (Batty and Smith, 2010). Hemostasis is a complex, stepwise process divided into primary and secondary hemostasis (Brass, 2003). There is substantial overlap between primary and secondary hemostasis as well as tight regulation of these processes to ensure that clot formation only occurs at the site of injury, and to prevent spontaneous thrombosis (Batty and Smith, 2010).

2.2.1 Primary Hemostasis

Primary hemostasis begins rapidly upon blood vessel injury. The main outcomes of primary hemostasis are vasoconstriction, to limit blood flow to the affected area, and initiating the formation of the platelet plug (Hiller, 2007). Specifically, damage to the endothelial cells of the injured vessel triggers a switch from the release of vasodilatory compounds (e.g., NO and prostacyclin) to compounds that promote vasoconstriction (e.g., endothelin-1) (Hiller, 2007). This reduces blood flow to the site of injury and helps slow the rate of bleeding, ultimately ensuring that blood clotting is targeted to the site of vascular injury (Hiller, 2007). Moreover, intact endothelial cells release vWF and tissue factor, which facilitate platelet tethering and initiation of the coagulation cascade at the site of injury, respectively (Periayah et al., 2017).

Initiation of the platelet plug occurs when vWF binds to subendothelial collagen, which is exposed following endothelial cell damage (Periayah et al., 2017). vWF is critical in scenarios of high shear stress, as it provides an initial tether for platelets that are quickly passing by the site of vessel injury (Sadler, 1998). Then, two platelet integrins (α2β1 and αIIbβ3) are rapidly activated by the platelet immunoglobulin receptor GPVI, which is activated by collagen (Lecut et al., 2004). This allows the platelets to form stable interactions directly with the exposed subendothelial collagen (Yun et al., 2016), which is strengthened by interactions with P-selectin (Merten et al., 2000, 1999). This interaction triggers platelet activation resulting in a conformational change (i.e., from a round shape to a star-like shape), increasing the surface area of the platelet and further strengthening the interaction between the platelet and the subendothelial surface (Yun et al., 2016).

Moreover, this conformational change can trigger the release of the granular content from the platelets including strong and weak platelet agonists (e.g., thrombin, thromboxane A_2 and ADP) that further encourage platelet activation (Yun et al., 2016) (Figure 2-3). These platelet agonists and their corresponding receptors will be discussed in detail in a subsequent section. Additionally, platelets are capable of capturing and storing circulating serotonin, which is released with its conformational change (Mercado and Kilic, 2010). Serotonin has been shown to play a role in vasoconstriction at the site of injury and can further promote platelet aggregation by providing a positive feedback loop on the release of platelet granular content (Mercado and Kilic, 2010).

Extension of the platelet plug occurs when activated platelets begin to aggregate on top of the initial monolayer of platelets bound to collagen. Platelet surface receptors and the accumulation of platelet agonists are critical to this response. Additionally, activation of the integrin αIIbβ3 is required to create stable fibrinogen and vWF-mediated bridges between platelets. Importantly, platelet activation results in phosphatidylserine moving from the inner platelet membrane to the outer membrane, which promotes the binding of coagulation factors, a step that is critical for strengthening the forming blood clot (Batty and Smith, 2010).

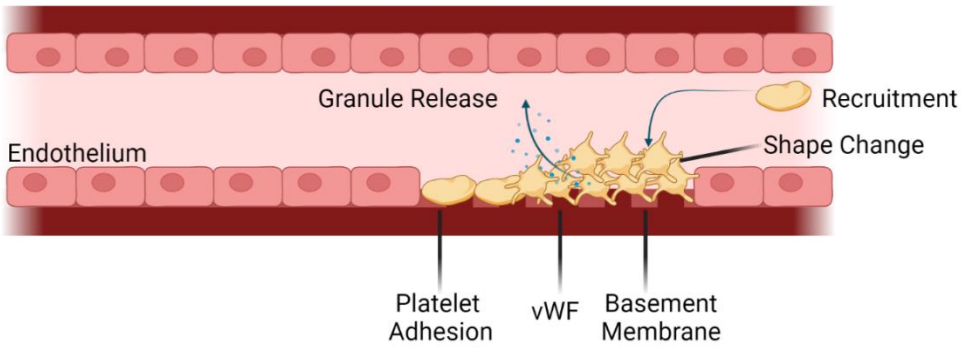

Figure 2-3: Simplified schematic depicting the steps in primary hemostasis.

Platelets adhere to the damaged basement membrane via interactions with von Willebrand factor (vWF). In addition, platelets are activated, release their granular content, and additional platelets are recruited to the site of vascular injury (Wickham, *unpublished*).

2.2.2 Secondary Hemostasis

Secondary hemostasis is characterized by the activation of coagulation factors as well as formation and stabilization of the fibrin network (Gale, 2011). The coagulation cascade, first described in the 1960s, highlights the sequential conversion of each coagulation factor to an active form, which results in the formation of a stable fibrin clot (Davie and Ratnoff, 1964; Macfarlane, 1964). The coagulation cascade is often subdivided into an intrinsic system, involving elements only found in blood, and an extrinsic system that relies on a tissue factor for activation (Gale, 2011) (Figure 2-4).

2.2.2.1 Intrinsic Pathway

The intrinsic pathway is activated when Factor XII binds to a negatively charged surface (e.g., exposed collagen in the subendothelium). Factor XII undergoes a conformational change that makes it highly susceptible to proteolytic cleavage by plasma kallikrein, a potent protease (Schmaier, 2008). Once proteolyzed, Factor XII becomes Factor XIIa, which facilitates the proteolysis of Factor XI to Factor XIa (Palta et al., 2014). Then, Factor

XIa triggers the proteolysis of Factor IX to Factor IXa, which acts with Ca^{2+}, Factor VIII, and platelet phospholipid 3 to convert Factor X into Factor Xa. Notably, this is where the common pathway between intrinsic and extrinsic coagulation begins. Factor Xa, Ca^{2+}, platelet phospholipid, and Factor V convert Factor II (i.e., prothrombin) into thrombin, which is the enzyme responsible for converting Factor I (i.e., fibrinogen) into fibrin. Stabilization of these monomers occurs via the formation of covalent bonds, termed crosslinks, between fibrin monomers (Palta et al., 2014). Activated Factor XIII is the enzyme responsible for this interaction and is activated by thrombin and Ca^{2+} (Figure 2-4). Thrombin release results in a positive feedback loop that causes further activation of Factors V and XIII as well as platelets (Palta et al., 2014).

2.2.2.2 Extrinsic Pathway

A critical component for activation of the extrinsic pathway of blood coagulation is tissue factor, which is a transmembrane receptor for Factor VII/VIIa. Under normal conditions, tissue factor is a surface protein found on cells surrounding blood vessels and is physically separated from Factor VII/VIIa via the endothelium (Mackman, 2009). However, once damage or trauma occurs to the endothelial barrier, tissue factor is exposed to Factor VII/VIIa and they form a complex which interacts with Ca^{2+} and platelet phospholipids to activate Factor X (Palta et al., 2014) (Figure 2-4). Then, the common coagulation cascade occurs.

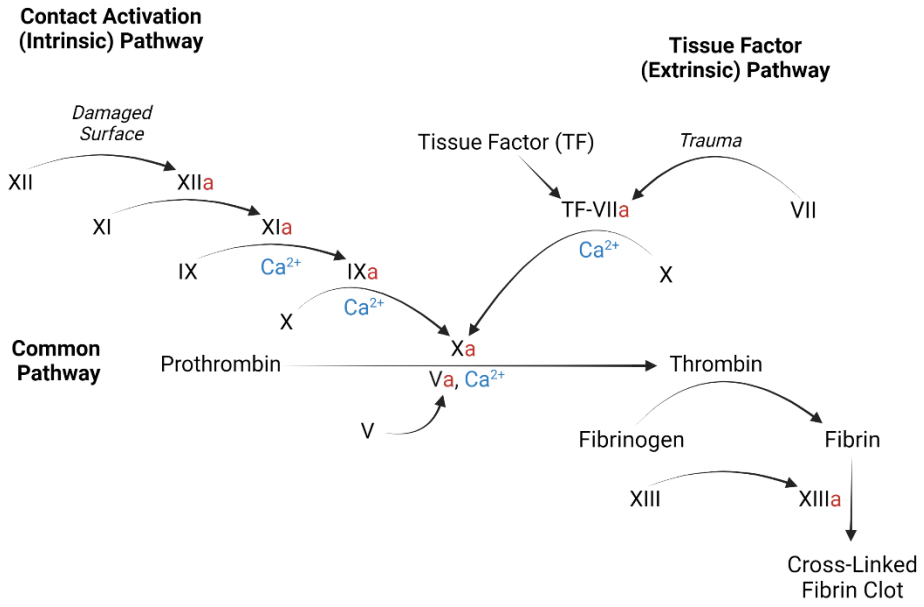

Figure 2-4: The coagulation cascade.

This cascade consists of an intrinsic pathway initiated by factors in the blood and an extrinsic pathway activated by tissue factor (TF) (Wickham, *unpublished*).

2.2.3 Platelet Agonists and Platelet Receptors

Platelet function is driven by the interaction of physiological agonists with receptors on the platelet membrane (Phillips, 1985). Platelet agonists are often classified by their strength of action, as strong (e.g., collagen, thrombin, thromboxane A_2) and weak (e.g., ADP, epinephrine) agonists. Strong platelet agonists promote the conformational change and aggregation of platelets as well as the secretion of the storage granules. Conversely, weak platelet agonists only trigger the conformational change and subsequent platelet aggregation (Phillips, 1985). Key mediators of platelet aggregation in human physiology are discussed below.

2.2.3.1 Collagen

Platelets express a variety of collagen receptors. As discussed previously, the integrins α2β1 and αIIbβ3 are important for platelet adhesion to exposed subendothelial collagen

(Periayah et al., 2017). Additionally, glycoprotein VI (GPVI) is a receptor required for collagen-induced platelet activation, as it is involved in signalling cascades that increase $[Ca^{2+}]_i$, which promotes platelet activation by inducing a conformational change and granule secretion (Brass, 2003). There are redundancies in platelet $[Ca^{2+}]$ signalling, as $[Ca^{2+}]_i$ increases in response to several platelet agonists.

2.2.3.2 Thrombin

Thrombin is a byproduct of the coagulation cascade and contributes to a positive feedback loop promoting further platelet activation (Brass, 2003). Thrombin activates protease-activated receptors (PAR) located on the platelet membrane (e.g., PAR-1 and PAR-4), ultimately initiating signalling cascades that increase $[Ca^{2+}]_i$ and inhibit cyclic adenosine monophosphate (cAMP) formation. The inhibition of cAMP removes the inhibition of platelet activation (Brass, 2003). There are redundancies in platelet cAMP signalling, as it is inhibited by several platelet agonists.

2.2.3.3 Thromboxane A_2

Thromboxane A_2 is formed within the platelet via the conversion of arachidonic acid to prostaglandin H_2 via cyclooxygenase-1. Then, prostaglandin H_2 is converted to thromboxane A_2 via thromboxane A_2 synthase (Hecker and Ullrich, 1989). Released thromboxane A_2 can bind to platelet surface thromboxane A_2 receptors. Like thrombin and PAR activation, thromboxane A_2-induced platelet activation results in increased $[Ca^{2+}]_i$ and inhibited cAMP formation (Z. Li et al., 2010).

2.2.3.4 Adenosine 5'-Diphosphate

ADP is released from within platelets as well as from the damaged endothelial cells (Woulfe et al., 2001). ADP initiates platelet aggregation via $P2Y_{12}$ and $P2Y_1$ membrane-bound receptors (Woulfe et al., 2001). Activation of the $P2Y_1$ receptor both increases $[Ca^{2+}]_i$ and inhibits cAMP formation (Jin et al., 1998). Conversely, activation of the $P2Y_{12}$ receptor only inhibits cAMP formation (Dorsam and Kunapuli, 2004).

2.2.3.5 Epinephrine

Epinephrine is released into the circulation from the adrenal glands and can bind to α_2-adrenoreceptors located on the platelet membrane (Hoffman et al., 1982). Epinephrine has been shown to reduce cAMP levels, which promotes platelet activation (Spalding et al., 1998).

2.2.4 Fibrinolysis

Fibrinolysis is the process of converting plasminogen to plasmin, which can bind to and dissolve fibrin blood clots (Chapin and Hajjar, 2015). Specifically, plasminogen activator mediates this conversion, and is produced in endothelial cells and then released into the circulation, where it is immediately bound to a plasminogen activator inhibitor (Troy, 1988). Plasminogen activator only becomes activated once it is bound to the fibrin network of a blood clot, and the plasminogen activator inhibitor is released back to the circulation (Troy, 1988). Therefore, the conversion of plasminogen to plasmin occurs locally on the fibrin network of the blood clot (Troy, 1988). Additionally, although to a lesser extent, Factor XIIa and kallikrein can also covert plasminogen to plasmin.

Plasmin directly targets fibrinogen to release several fibropeptides, eventually forming two small fragments called “D” and “E” that have anti-coagulant and anti-platelet effects (Weisel and Litvinov, 2017). Plasmin can also target fibrin to degrade clots. All degradation products are removed from the circulation by the liver (Troy, 1988). Once the clot is degraded, plasmin is released back into the circulation and immediately inactivated by interactions with plasmin inhibitors (e.g., α2-antiplasmin and α2-macroglobulin) (Rosenberg and Rosenberg, 1984).

The long-term solution for chronic vascular inflammation and injury is to recruit fibroblasts to the damaged area, which produce and release collagen, to create a strong repair at the damaged site (Li and Wang, 2011). This process, termed fibrosis, progresses from an initially reversible state to permanent changes in the structure (e.g., arterial stiffening) and function (e.g., endothelial function) of the vasculature, ultimately contributing to the development of cardiovascular disease (Huveneers et al., 2015).

2.2.5 Arterial Thrombosis

If an imbalance between clot formation and degradation occurs, large blood clots that are not sufficiently degraded may trigger severe thrombotic events including arterial thrombosis, a principal cause of heart attack and stroke (Wilkerson and Sane, 2002). As described previously, platelet activation is a critical factor in the initiation of arterial blood clot formation (Periayah et al., 2017) and platelets and fibrin are the main constituents of the clot (Gale, 2011). Notably, the risk of arterial thrombosis is significantly increased in scenarios where the arteries are narrowed and endothelial function is impaired, such as fibrosis or atherosclerosis (Olvera Lopez et al., 2022).

2.3 Sex Hormones in Women

Briefly, the endocrine system plays a role in controlling reproductive hormone production, and the regulation of reproductive hormones begins in the brain (Marrocco and McEwen, 2016). Specifically, the hypothalamus is responsible for producing gonadotropin-releasing hormone, which stimulates the pituitary gland to release gonadotropins (Harris, 1948). In women, the gonadotropins (i.e., luteinizing hormone and follicle-stimulating hormone) are found in the ovaries and facilitate the production of estrogens, progestogens, and testosterone (Marrocco and McEwen, 2016). These hormones are fundamental to reproductive development (Ojeda et al., 2003) and function as well as acting as signalling molecules in other tissues of the body (Fuentes and Silveyra, 2019). This dissertation will focus on the role of estrogens with regards to vascular health.

2.3.1 Estrogens

Estrogens are steroid sex hormones produced predominantly in the ovaries of women (Hamilton et al., 2017). Estrogens are found in three physiological forms: estradiol, estrone, and estriol (Lievertz, 1987). Notably, estradiol is the most potent and abundant form of circulating estrogen. In young, healthy women estradiol levels fluctuate throughout the menstrual cycle, where the highest levels of estradiol occur near ovulation in the late follicular phase (Reed and Carr, 2018). Circulating estradiol levels are also affected by life stage, where estradiol levels rise through adolescence, peaking in adulthood, and with a gradual decline until the menopausal transition, which is associated with a cessation of endogenous production of estradiol (Horstman et al., 2012) (Figure 2-5). Estrogens, particularly estradiol, are known to play key roles in regulating physiological processes (Fuentes and Silveyra, 2019), and its pivotal role in the cardiovascular system is addressed in the next section.

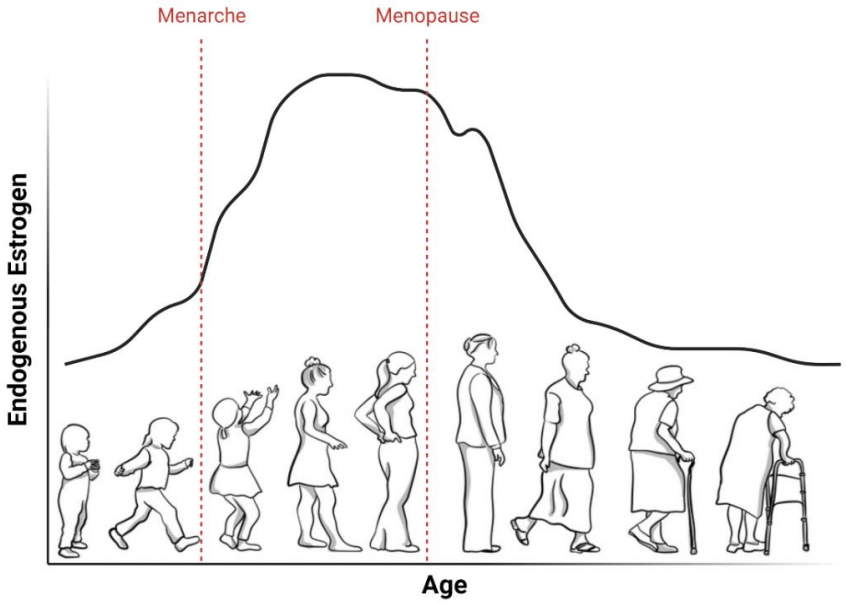

Figure 2-5: Endogenous estrogen levels throughout the lifespan in women. Notably, estrogen primarily refers to estradiol (Wickham, unpublished*).*

2.3.2 Progestogens

Progestogens are another steroid sex hormone produced predominantly in the ovaries of women (Prior, 2020). Progesterone is the main progestogen in women (Prior, 2020). Progesterone levels fluctuate throughout the menstrual cycle in young, healthy eumenorrheic women, where progesterone levels are highest after ovulation in the mid-luteal phase (Reed and Carr, 2018). Aging is associated with a decline in endogenous progesterone, and menopause results in a withdrawal of endogenous progesterone production (Welt et al., 1999). However, little is known regarding the physiological impact of the loss of progesterone in aging women.

2.3.3 Androgens

In women, androgens are primarily synthesized in the adrenal glands and the ovaries (Burger, 2002). Testosterone is the predominant androgen in both men and women (Burger, 2002). In women, circulating testosterone levels gradually decline with age and menopause (Brzozowska and Lewiński, 2020). Interestingly, around menopause, the androgen:estrogen ratio significantly increases, as the ovaries are still able to produce androgens for up to 10 years after menopause whereas estrogen production ceases (Fogle et al., 2007; Markopoulos et al., 2015). However, it is unclear how the androgen:estrogen relationship impacts the physiological consequences of menopause.

2.4 Estrogen Receptor Interactions and the Cardiovascular System

Healthy women in their reproductive years are at a significantly lower risk of developing cardiovascular disease than healthy age-matched men (Bittner, 2009). A statistic that is

often attributed to the cardioprotective effects of estradiol (El Khoudary et al., 2020), which are achieved through a complex array of signalling pathways and biochemical reactions that alleviate oxidative stress (Miller and Duckles, 2008). This is critical as oxidative stress can damage tissues of the body and is considered a key mechanism in vascular aging (Izzo et al., 2021; Liguori et al., 2018). This section will briefly highlight the roles and mechanisms of estrogens in mitigating oxidative stress.

2.4.1 Genomic Estrogen Receptor Signalling

In the circulation, estrogens are bound to estrogen binding proteins. Since estrogens are lipid soluble molecules, they can freely cross the plasma membrane of cells and bind to estrogen receptors (i.e., estrogen receptor α (ERα) and estrogen receptor β (ERβ)). Although estrogens typically interacts with cytosolic estrogen receptors, 5-10% of estrogen receptors are located on the plasma membrane (Levin, 2009). Notably, 17β-estradiol has an equal binding affinity for both ERα and ERβ (Kuiper et al., 1997). The binding of estrogens to their receptor stimulates dimerization of two estrogen-receptor complexes, and translocation of the complexes to the nucleus of the cell, where the complex activates estrogen response elements (ERE) (Nadal et al., 2001). This ultimately triggers the upregulation or downregulation of protein expression (McDonnell and Norris, 2002) (Figure 2-6). Critical to vascular health, estrogens can increase eNOS, VEGF, and superoxide dismutase 2 (SOD2) protein expression, which help combat oxidative stress (Miller and Duckles, 2008).

Orphan estrogen-related receptors (ERR) are nuclear receptors that possess chemical structures similar to the estrogen receptors (Liu et al., 2003). The ERR family is comprised

of ERRα, ERRβ, and ERRγ (Xia et al., 2019). Interestingly, ERR are considered "orphan" receptors because they currently do not have an identified endogenous ligand (Tripathi et al., 2020). Therefore, despite their similarity to estrogen receptors, ERR are not activated by estrogens or estrogen-like molecules (Villena and Kralli, 2008). This dissertation will focus on the importance of ERRα, which plays key roles in metabolically active tissues, such as skeletal muscle (Villena and Kralli, 2008). ERRα is activated by interactions with peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), often termed the master regulator of mitochondrial biogenesis (Laganière et al., 2004), and has been identified as a primary regulator of the expression of proteins related to mitochondrial energy production (Schreiber et al., 2004; Tripathi et al., 2020). Importantly, ERRα may play important roles in promoting cardiovascular health, particularly in the context of the mechanisms underpinning cardiovascular adaptations to exercise training. This will be discussed in detail in a subsequent section.

2.4.2 Non-Genomic Estrogen Receptor Signalling

Estrogens can also bind to the plasma membrane-bound G protein-coupled estrogen receptor (GPER), which when activated can post-translationally modify target proteins (Prossnitz and Barton, 2011). For example, GPER activation can facilitate eNOS activation and NO production via increased $[Ca^{2+}]_i$ (Fredette et al., 2018) (Figure 2-6).

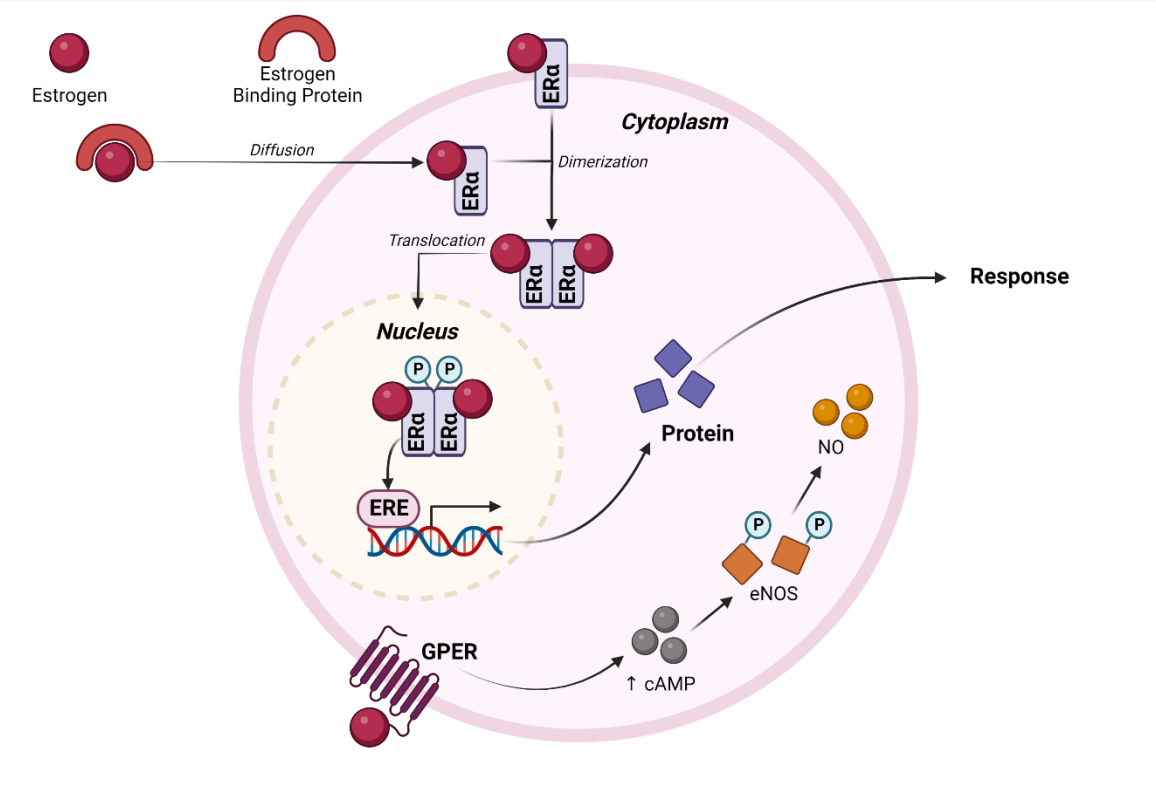

Figure 2-6: Genomic and non-genomic estrogen receptor pathways.

Genomic signalling pathways affect protein expression by altering the transcription of proteins (e.g., ERα) and non-genomic pathways induce post-translational modifications to alter protein activity (e.g., GPER). ERα; estrogen receptor alpha, ERE; estrogen response element, GPER; G protein-coupled estrogen receptor, cAMP; cyclic adenosine monophosphate, eNOS; endothelial nitric oxide synthase, NO; nitric oxide. **Note:** Although not depicted in this schematic, estrogen binding to estrogen receptor β (ERβ) follows the same pathway (Wickham, *unpublished*).

2.4.3 Structural Significance of Estrogens

The biochemical structure of estrogens can elicit cardioprotective effects beyond the *classical* estrogen-mediated and estrogen-related receptor pathways. Specifically, estrogens have antioxidant properties due to its chemical structure, which features an aromatic ring with a free phenolic hydroxyl group. Importantly, the hydrogen ion from the hydroxyl group can be donated to scavenge free radicals (Badeau et al., 2005).

2.4.4 Ratio of ERα to ERβ

ERα is predominantly responsible for exerting the anti-inflammatory effects, while ERβ has been suggested to be related to a pro-inflammatory profile (Koenig et al., 2017; Novella et al., 2012). The loss of estrogen associated with the menopausal transition has been suggested to result in a reduction in ERα protein expression, decreasing the ratio of ERα:ERβ, and ultimately favouring a pro-inflammatory phenotype (Novensà et al., 2011; Park et al., 2017).

2.5 Aging, Menopause, and Vascular Health

The complex interplay between, and relative contribution of, aging and menopause on cardiovascular disease risk has been debated for more than 30 years (Bittner, 2009). Aging and menopause occur concurrently and are intimately linked processes, which makes teasing apart their contributions to cardiovascular disease risk complicated and challenging (Bittner, 2009).

2.5.1 Aging

Under healthy conditions, the tissues of the body are constantly undergoing a balance of damage and repair processes (Yousefzadeh et al., 2021). However, it is generally accepted that the ultimate cause of aging is inadequately combatted damage to the body's macromolecules (Yousefzadeh et al., 2021). Specifically, if cellular damage is not sufficiently repaired, it will accumulate and lead to physiological consequences (e.g., oxidation of deoxyribonucleic acid (DNA)). Cellular damage is largely attributed to the generation of reactive oxygen species, which react with DNA, proteins, and other

macromolecules in the body (Liguori et al., 2018). Accordingly, the ability to attenuate damage and the efficacy of the repair processes is critical in determining the rate of physiological aging (van Beek et al., 2016). However, changes in protein structure and other molecular interactions with proteins can also induce cellular damage (van Beek et al., 2016), and other biological processes contribute to aging (e.g., telomere shortening) (van Beek et al., 2016).

Notably, aging is linked to impairments in skeletal muscle strength and endurance (Izquierdo et al., 2001) as well as an increased susceptibility to disease development and progression (Franceschi et al., 2018). These factors can significantly compromise an individual's quality of life as well as health- and lifespan.

2.5.2 Healthy Human Aging

Human evolution highlights that the 'default' for health is to be physically active (Harridge and Lazarus, 2017). However, today, our society is facing a global health pandemic of physical inactivity (Kohl et al., 2012). Like a poor diet or smoking, physical inactivity poses significant negative physiological consequences that confound healthy human aging (Harridge and Lazarus, 2017). Importantly, sedentary behavior increases with advancing age (Kim and Lee, 2019). Therefore, it may be erroneous to study physically *inactive* older adults to understand the true physiological effects of aging. Instead, it has been suggested that studies should investigate physically active older adults, who are often devoid of other complicating health factors (e.g., obesity, diabetes, or other lifestyle-related diseases), to determine the inherent physiological impact of aging (Harridge and Lazarus, 2017). Findings from master athletes (i.e., competitive older adult athletes) demonstrate

that, despite maintaining good physical fitness, there is still a gradual linear decline in health and cardiorespiratory fitness until the eighth decade of life. Thereafter, health and performance decrements occur at an accelerated rate (Harridge and Lazarus, 2017). Accordingly, it has been proposed that a certain threshold of regular physical activity is required for optimal aging and to maximize an individual's health span (e.g., physical function and independence) (Lazarus and Harridge, 2017).

2.5.3 Vascular Consequences of Aging

Aging is an established risk factor for the development of cardiovascular disease, primarily attributed to vascular oxidative stress and subsequent vascular endothelial dysfunction (Seals et al., 2011). For example, peak forearm blood flow stimulated by the endothelium-dependent vasodilator acetylcholine declines progressively with increasing age (DeSouza et al., 2000). Similarly, brachial artery flow-mediated dilation (FMD), a gold standard evaluation of conduit artery function, is impaired with age (Celermajer et al., 1994). Moreover, there has been interest in the differences between conduit artery function in the arm versus the leg. This is attributed to differences in the orthostatic challenges on the vasculature, where the legs may be protected to a greater extent due to increased shear stress from walking. However, it has been shown in healthy older adults that leg FMD is also impaired with age (Nishiyama et al., 2008).

Interestingly, individuals who are classified as lifelong physically active have been shown to have a lower risk of developing cardiovascular disease (Maessen et al., 2016). This may be attributed an attenuation of age-related declines in cardiovascular function with lifelong exercise training compared to physically inactive or low activity individuals

(Ozemek et al., 2018). Notably, lifelong participation in exercise has been linked to reduced arterial stiffness (Shibata et al., 2018) and enhanced NO bioavailability (Nyberg et al., 2012) compared to physically inactive controls. Unfortunately, comprehensive longitudinal studies on vascular health and function in lifelong active individuals are lacking, making it difficult to comment directly on the age-specific impact, as lifelong physically active individuals may also be less likely to have poor lifestyle habits (e.g., diet, smoking, etc.) compared to physically inactive individuals.

2.5.4 Menopause and the Hormonal Milieu

On average women enter menopause at the age of ~50 years old, with most women experiencing menopause between the ages of 45 and 55 (Hinchliff, 2019). Therefore, amidst increasing life expectancies worldwide, the average woman will live 30 to 40% of their life postmenopausal.

The menopausal transition denotes a break in the regular menstrual rhythm, triggered by a diminishing oocyte supply (Santoro and Randolph, 2011). The menopausal transition is defined by two stages, early and late. The early phase of the menopausal transition is characterized by skipped menstrual cycles or irregularities in cycle duration (>6 days) (Harlow et al., 2008). The early menopausal transition is terminated, and the late menopausal transition stage begins, when the oocyte supply reaches critically low levels, and a woman has been amenorrheic for >60 days (Harlow et al., 2008). Menopause is defined as ≥12 months without menstrual bleeding (Wallace et al., 1979). The menopausal transition and first few years of menopause are accompanied by several symptoms including, but not limited to, hot flashes, insomnia, joint pain, and fatigue

(Santoro et al., 2015). These symptoms are attributed to drastic changes in the hormonal milieu throughout the menopausal transition and, more than 85% of women will experience at least one of these menopausal symptoms (Kalhan et al., 2020).

There are several key blood markers of the menopausal transition including follicle-stimulating hormone (Randolph et al., 2011), anti-Mullerian hormone (Sowers et al., 2008), inhibin B (Danforth et al., 1998), and estradiol (Finkelstein et al., 2008). Notably, anti-Mullerian hormone and inhibin B are produced by early ovarian follicles, making them direct reflections of the ovarian follicular pool. Specifically, as the number of ovarian follicles declines with age, anti-Mullerian hormone and inhibin B levels also decline (Danforth et al., 1998; Sowers et al., 2008). However, due to its widespread availability and low cost of analysis, follicle-stimulating hormone is the most common marker of the menopausal transition (Santoro and Randolph, 2011). Follicle-stimulating hormone is produced by the hypothalamus in the brain and is typically responsible for stimulating ovulation. However, with increasing age and a shift towards the menopausal transition, the ovaries no longer respond to normal follicle-stimulating hormone levels. Therefore, more follicle-stimulating hormone is released in an attempt to trigger ovulation. This rise in follicle-stimulating hormone is a hallmark of the menopausal transition ($\geq$30 $IU \cdot L^{-1}$), which remains elevated in menopause (Randolph et al., 2011). Lastly, in premenopausal women estradiol levels range between 30 to 4000 $pg \cdot mL^{-1}$ and are largely maintained throughout the early menopausal transition (Su and Freeman, 2009). However, after menopause, estradiol levels decrease dramatically to <30 $pg \cdot mL^{-1}$ (Finkelstein et al., 2008). Importantly, it is typically the decline in endogenous estradiol levels that is

associated with adverse health outcomes in postmenopausal women (Iqbal and Zaidi, 2009).

2.5.5 Vascular Consequences of Menopause

Men demonstrate a relatively steady rise in arterial blood pressure with age. Conversely, women have largely unaltered blood pressure until menopause, thereafter a relatively rapid rise is observed (Barton and Meyer, 2009; Staessen et al., 1997). Similarly, in both men and women there is a gradual decline in vascular function with aging, however in women this decline is significantly more pronounced after menopause (Holder et al., 2019), suggesting that these impairments are linked to the loss of endogenous estrogen associated with menopause. Accordingly, an interest has been sparked in understanding the accelerated vascular aging that occurs with the loss of endogenous estrogen at menopause.

An intriguing prospective study by Matthews et al., (2009) followed 1054 late premenopausal women for one year before their last menstrual bleeding as well as for the year after their last menstrual bleeding. They found that total cholesterol, low density lipoproteins, and apolipoprotein B (i.e., blood lipids) were substantially elevated within the year after their last menstrual bleeding (Matthews et al., 2009). This study provided preliminary evidence that even the menopausal transition can elicit significant effects on factors that influence cardiovascular health. In recent years, researchers have continued to elucidate the contribution of menopause to cardiovascular disease risk by comparing late premenopausal women and recently postmenopausal women, two cohorts that only differ marginally in age (e.g., <5 years). Notably, recently postmenopausal women display

characteristics of impaired cardiovascular health including elevated diastolic blood pressure, markers of vascular inflammation, thromboxane A_2 synthase (important for vasoconstriction and platelet aggregation) (Nyberg et al., 2014), total cholesterol (Mandrup et al., 2017), and basal platelet reactivity (Lundberg Slingsby et al., 2017) compared to late premenopausal women. Together, this evidence suggests that the first few years of menopause pose a significant threat to cardiovascular health.

2.6 Menopause and Hormone Replacement Therapy

Hormone replacement therapy (HRT) involves administering exogenous sex hormones to postmenopausal women to relieve symptoms of menopause as well as mitigate disease risk associated with menopause (Harper-Harrison and Shanahan, 2022). The first HRT treatments utilizing estrogen replacement only were produced as early as the 1900s, but only became an established treatment option for postmenopausal women in the 1940s. Estrogen-only HRT first gained widespread popularity during the feminist movement of the 1960s, and combined estrogen and progestogen HRT gained substantial popularity in the 1980s and 1990s (Rosano et al., 2009). Accordingly, the first clinical trials on combined estrogen and progestogen HRT were conducted in the United States in the early 1990s (Nelson et al., 2002; Rossouw et al., 2002).

2.6.1 Administration of Hormone Replacement Therapy

There are a wide variety of HRT strategies featuring different hormonal combinations and administration strategies. Most women using HRT will take a combination of estrogen and progestogen. However, in women who have had a hysterectomy (i.e., surgical removal of

the uterus), which artificially induces menopause, estrogen-only therapy can be prescribed (Haney and Wild, 2007). Exogenous estrogens can be administered orally, transdermally via patches, gels, sprays, or subcutaneous implants, vaginally, or by injection (Harper-Harrison and Shanahan, 2022). Alternatively, progestogens can only be administered orally or transdermally (Harper-Harrison and Shanahan, 2022). Therefore, combined HRT is delivered either orally or transdermally. HRT can be taken daily or used in cycles (Harper-Harrison and Shanahan, 2022).

2.6.2 Proposed Benefits of Hormone Replacement Therapy

In the 1990s and early 2000s, several studies emerged showing that HRT could alleviate menopausal symptoms and improve the health and lifespan of postmenopausal women (Hackley and Rousseau, 2004). For example, HRT has been shown to significantly reduce the severity of hot flashes (Greendale et al., 1998), insomnia (Polo-Kantola et al., 1999), and genitourinary symptoms (Willhite and O'Connell, 2001). Notably, women with a history of estrogen therapy (Henderson et al., 1991) and combined HRT (Grodstein et al., 1997) use had lower all-cause mortality than non-users. Other studies have demonstrated a reduced risk of developing colorectal cancer (Chlebowski et al., 2004), dementia (Yaffe et al., 1998), cardiovascular disease (Grady et al., 1992), and bone fractures (Cauley et al., 2003) among HRT users.

2.6.3 Adverse Effects Associated with Hormone Replacement Therapy

Despite the initial glowing reviews of HRT, over the last few decades HRT has become one of the most controversial topics related to women's health, as a mounting body of evidence points towards a multitude of adverse side effects associated with HRT. Initial

concerns arose in 1975 when evidence began to show that estrogen-only HRT was associated with an increased risk of developing endometrial cancer (Smith et al., 1975; Ziel and Finkle, 1975). Approximately a decade later, the initial unease subsided, and the popularity of HRT skyrocketed once the United States Food and Drug Administration approved the use of combined estrogen and progestogen HRT in 1988 (Cagnacci and Venier, 2019). However, additional concerns quickly arose from the first clinical trial titled the Women's Health Initiative conducted in the United States in 2002. The HRT arm of the study was terminated prematurely due to safety issues including the development of various types of cancer as well as cardiovascular disease (Nelson et al., 2002; Rossouw et al., 2002). Supporting these findings, a recent meta-analysis showed that postmenopausal women using HRT are at an increased risk of developing ovarian cancer compared to untreated postmenopausal women (Liu et al., 2019). Moreover, evidence from large-scale randomized HRT trials showed that long-term use (i.e., >5 years) increased the risk of developing breast cancer (Kerlikowske et al., 2003; Li et al., 2003; Marsden, 2002). Interestingly, this risk declines following the cessation of HRT treatment, suggesting a tumour growth promoting effect of HRT (Fournier et al., 2014).

It appears that there is a 'timing effect' and 'health status effect' when considering the impact of HRT on cardiovascular disease risk in postmenopausal women. First, HRT is associated with significantly greater adverse cardiovascular health effects if initiated late after the beginning of menopause (i.e., >10 years) (Goldman, 2004; Rossouw et al., 2007). This evidence stems from a revisit to The Women's Health Initiative data, which unveiled that HRT was only beneficial for postmenopausal women <10 years into menopause,

whereas increased health risks were observed in the postmenopausal women initiating HRT more than 10 years after the start of menopause (Hsia et al., 2006; Rossouw et al., 2007). Additional follow up studies have narrowed this window by showing HRT-induced improvements in blood pressure, plasma catecholamine, and cholesterol levels in women <5 years after menopause, whereas there was no benefit in the women ≥5 years after menopause (Brownley et al., 2004). Second, HRT should only be initiated in healthy postmenopausal women, as some evidence suggests that women with increased cardiovascular disease risk experienced an increased number of cardiovascular events during the first year of HRT despite positive effects on markers of cardiovascular health including lowered total cholesterol, elevated high-density lipoproteins, improved vasodilation, and reduced inflammation (Joswig et al., 2000). Alternatively, a series of studies have reported no beneficial effect of HRT on the occurrence of cardiovascular events in postmenopausal women with existing cardiovascular disease (Cho and Mukherjee, 2005).

2.7 Dual Anti-Platelet Therapy (DAPT) and Thrombotic Risk

Acute coronary syndrome, characterized by sudden reduced blood flow to the heart, is typically triggered by an atherosclerotic plaque rupture and subsequent thrombosis, whereby a blood clot obstructs flow through a vessel (Alkarithi et al., 2021). Following a non-fatal acute coronary syndrome, patients are prescribed dual anti-platelet therapy (DAPT) for 12 months (Collet et al., 2021) to reduce platelet hyperreactivity and the formation of harmful blood clots by dramatically increasing the anti-platelet effects of NO and prostacyclin (Chan et al., 2016; Collet et al., 2021; Kirkby et al., 2013; Warner et al.,

2016). DAPT is a daily medication consisting of aspirin and a $P2Y_{12}$ receptor antagonist (e.g., ticagrelor or clopidogrel), which synergistically inhibit platelet activation (Manolis et al., 2013).

2.7.1 Aspirin

Aspirin, also known as acetylsalicylic acid, inhibits platelet aggregation by irreversibly inhibiting cyclooxygenase-1, an enzyme that is important for the conversion of arachidonic acid to prostaglandin H_2 and ultimately thromboxane A_2 production via thromboxane A_2 synthase (Warner et al., 2011) (Figure 2-7). However, prostaglandin H_2 is a crucial control point in platelet metabolism as it can also be converted to prostacyclin, an inhibitor of platelet aggregation, via prostacyclin synthase. Accordingly, via cyclooxygenase-1, it is impossible to inhibit thromboxane A_2 production without simultaneously affecting prostacyclin production. Therefore, it has been important to find the optimal dosing regimen to maximize the inhibition of thromboxane A_2, while minimizing the impact on prostacyclin. Recent evidence suggests that low doses of aspirin (i.e., <80 $mg \cdot day^{-1}$; equivalent to one tablet of baby aspirin) elicit an optimal anti-platelet response (i.e., ≥95% inhibition of platelet thromboxane A_2 production) (Parker et al., 2019), while prostacyclin production is affected to a lesser extent (FitzGerald et al., 1983; Thorp et al., 1988).

2.7.2 $P2Y_{12}$ Receptor Antagonists

The most common $P2Y_{12}$ receptor antagonists include clopidogrel (2^{nd} generation), prasurgrel (3^{rd} generation), and ticagrelor (4^{th} generation) (Warlo et al., 2019). Clopidogrel is a member of the thienopyridine family, which require metabolic activation by the liver

to provide anti-platelet effects. Clopidogrel elicits its anti-platelet effects through direct and irreversible binding to the ADP binding site on platelets (Savi et al., 1998). Prasurgrel is also a member of the thienopyridine family and affects platelet aggregation in the same way as clopidogrel, however the anti-platelet effects of prasurgrel are ~10-fold more potent (Norgard and Abu-Fadel, 2009). Conversely, ticagrelor belongs to the cyclopentyltriazolopyrimidine family and works by reversibly and non-competitively binding to the $P2Y_{12}$ receptor at a site that is distinct from the endogenous ADP binding site (Dobesh and Oestreich, 2014) (Figure 2-7). Ticagrelor has been associated with the best cardiovascular outcomes in patients with acute coronary syndrome (Briasoulis et al., 2016). Typically, ticagrelor is administered as 90 mg taken twice daily (Fuller and Chavez, 2012).

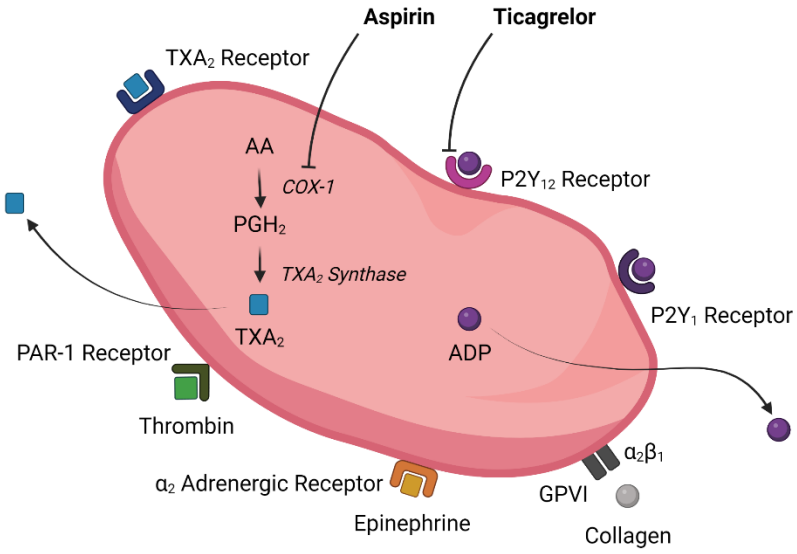

Figure 2-7:Dual anti-platelet therapy (DAPT) and platelet function.

Inhibition of platelet activation by treatment with dual anti-platelet therapy (DAPT) consisting of aspirin and a $P2Y_{12}$ receptor antagonist, such as Ticagrelor. AA; arachidonic acid, COX-1; cyclooxygenase-1, PGH_2; prostaglandin H_2, TXA2; thromboxane A_2, ADP; adenosine 5'-diphosphate; Ca^{2+}; calcium, GPVI; glycoprotein VI (Wickham, *unpublished*).

2.7.3 Efficacy of Dual Anti-Platelet Therapy (DAPT)

Since the 1950s, aspirin has been known to have anti-thrombotic effects (Miner and Hoffhines, 2007). However, it was not until the late 1980s and early 1990s that $P2Y_{12}$ receptor antagonists were first utilized for anti-platelet effects (Balsano et al., 1990; Gent et al., 1989; Hass et al., 1989). Accordingly, the first DAPT studies and clinical trials assessing recurrence of thrombotic events only emerged in the mid-to-late-1990s (Bertrand et al., 2000; Bossavy et al., 1998; Gregorini and Marco, 1997; Lablanche et al., 1996). Since then, numerous studies have confirmed the potent anti-platelet effects of DAPT and the prevention of thrombotic events (Elmariah et al., 2015; Lee et al., 2020; Xu et al., 2021), and recent investigations have attempted to optimize the dosing strategies and time course of treatment (Breet et al., 2011; Briasoulis et al., 2016; Mallidi and Lata, 2019; Trifan et al., 2021; Verdoia et al., 2021).

A recent meta-analysis showed that DAPT consisting of clopidogrel and aspirin, compared to aspirin alone, resulted in a reduction in recurrent vascular events 3 months after an initial stroke (Lee et al., 2020). Moreover, another meta-analysis highlighted that compared with monotherapy (i.e., one of either aspirin or a $P2Y_{12}$ receptor inhibitor), DAPT significantly reduced the risk of recurrent stroke, but significantly increased the risk of major bleeding (Trifan et al., 2021). However, when DAPT was administered as aspirin plus ticagrelor, the risk of major bleeding was comparable to monotherapy (Trifan et al., 2021). Together, these findings support the notion that DAPT is more effective than monotherapy, and DAPT consisting of aspirin and ticagrelor is the safest and most effective treatment strategy following acute coronary syndrome.

A previous study tested the efficacy of DAPT treatment for 12 vs. 30 months (Mauri et al., 2014). Although DAPT treatment for 30 months resulted in further reductions in rates of thrombosis and other cardiovascular events, the rate of all-cause mortality was 2.0% higher than the group that only received 12 months of treatment (Mauri et al., 2014). These findings have helped shape the guidelines promoting DAPT utilization for 12 months following an acute coronary syndrome. However, there are many inter-individual considerations determining the efficacy and length of DAPT treatment. For example, a recent meta-analysis suggests that very short-term DAPT followed by $P2Y_{12}$ receptor antagonist monotherapy may also be beneficial solution for patients with an increased risk of major bleeding (Xu et al., 2021; Zhong et al., 2021).

Taken together, both HRT and DAPT may prove beneficial as short-term interventions for reducing an individual's cardiovascular risk. However, when applied for longer durations they may be associated with adverse health outcomes, supporting the notion that safe and sustainable alternative therapies are required for the long-term maintenance of vascular health in postmenopausal women.

2.8 Menopause, Exercise Training, and Vascular Health

For the last few decades, exercise has been at the forefront of alternative therapies for attenuating age- and disease-related health decrements (Reuter, 2012). Exercise provides a potent physiological stress that activates signalling cascades which promote beneficial adaptations to exercise training (Reuter, 2012).

2.8.1 Exercise is Medicine

The *Exercise is Medicine* initiative was founded in 2007 by the American College of Sports Medicine, with the aim of making regular physical activity a known health promoting intervention (Thompson et al., 2020). Exercise is a "medicine" that can be used in the treatment and prevention of age-related health impairments and chronic disease, and there is a large body of evidence supporting that those who "take it" live longer and higher quality lives (Thompson et al., 2020). Moreover, it has been shown that physical activity can be as effective as pharmaceutical interventions in the secondary prevention of cardiovascular disease and diabetes (Naci and Ioannidis, 2013). Therefore, exercise provides a sustainable, cost-effective, and long-term alternative to promote cardiovascular health in all cohorts.

2.8.2 Exercise Training and Menopause

Exercise poses numerous health benefits for postmenopausal women including prevention of cardiovascular and metabolic syndromes, strengthening bones, increasing muscle mass, and reducing symptoms of menopause (Grindler and Santoro, 2015). Moreover, regular physical activity dramatically reduces all-cause mortality in postmenopausal women compared to sedentary postmenopausal women (Grindler and Santoro, 2015). These findings are relatively new, as the study of exercise training in postmenopausal women has only gained significant traction over the last two decades, despite knowing that exercise training is beneficial for cardiovascular health in older men for over 60 years (Brown et al., 1957).

This dissertation will focus on the vascular benefits of exercise training in postmenopausal women. Previous evidence suggests that exercise elicits cardioprotective effects in postmenopausal women, as those who participated in <1 hour of moderate-to-vigorous activity per week had a 58% greater risk of developing cardiovascular disease compared to women who exercised >3.5 hours per week (Li et al., 2006). The cardioprotective effects of exercise occur through several mechanisms. For example, regular exercise has been shown to improve the plasma lipid profile via reductions in triglyceride, low density lipoprotein, and very low density lipoprotein levels while increasing high density lipoprotein (HDL) levels (Muscella et al., 2020). Thus, decreasing the risk of atherogenesis (Muscella et al., 2020). Additionally, the shear stress from regular exercise has been shown to increase the bioavailability of NO, which significantly enhances vascular vasodilatory capacity and reduces arterial blood pressure (Green et al., 2004). Lastly, regular exercise has been shown to enhance the activity of endogenous antioxidant defense systems as well as reduce free radical generation (Tofas et al., 2020).

Recently, a few studies have compared the effects of exercise training in late premenopausal women and recently postmenopausal women to determine if the menopausal transition affects the efficacy of exercise training adaptations. Exercise training improves fitness (Seidelin et al., 2017), reduces body mass and increases lean mass and bone mineral density (Mandrup et al., 2017; Seidelin et al., 2017), improves cardiovascular function via reduced diastolic blood pressure (Nyberg et al., 2014), and increased heart volume and strain (Egelund et al., 2017) to an equal extent between late premenopausal and recently postmenopausal women. However, in a study by Lundberg

Slingsby et al. (2017) basal platelet reactivity was only improved in late premenopausal women, with no effect of training in recently postmenopausal women. Together, these findings suggest that cardiovascular adaptations can be achieved if exercise training is initiated shortly before or after menopause, although the benefits of exercise training may be maximized before the menopausal transition. Recently, the scientific community has started to expand on this question by asking whether the number of years after menopause could significantly impact the adaptations to exercise training.

2.8.3 The Timing of Exercise Training in Menopause

Although regular exercise training provides significant cardiovascular adaptations in men of all ages, the effects in older women are less consistent. In line with the evidence for the efficacy of HRT, the *exercise timing hypothesis* suggests that exercise training may be more effective for inducing cardiovascular benefits in recently compared to late postmenopausal women (Gliemann and Hellsten, 2019). Recently postmenopausal women are typically defined as being ≤5 years after the menopausal transition, whereas late postmenopausal women are ≥10 years postmenopausal. The *exercise timing hypothesis* is supported by a small but growing body of evidence highlighting no improvement in cardiovascular function after training in late postmenopausal women or groups of postmenopausal women with a diverse age range (Moreau et al., 2013; Pierce et al., 2011; Spina et al., 1993). However, significant cardiovascular improvements are seen in recently postmenopausal women (Egelund et al., 2017; Nyberg et al., 2016, 2014).

Several potential mechanisms have been proposed to explain the *exercise timing hypothesis*: **(1)** changes in estrogen receptor and ERRα signalling, **(2)** the initial degree of

vascular impairment, as well as **(3)** imbalances in antioxidant defense systems (Gliemann and Hellsten, 2019). First, regular physical activity can mimic some of the effects of endogenous estrogens through the activation of ERRα, via skeletal muscle contractions, promoting the production of key proteins related to vascular health (e.g., eNOS, SOD2, and mitochondrial biogenesis) (Craige et al., 2016; Perry et al., 2014) (Figure 2-8). Therefore, before menopause estrogenic effects can be achieved by both endogenous estrogens and regular physical activity. However, the menopausal transition results in the loss of ERE activation via estrogens binding to estrogen receptors, thereby reducing the potential for cardiovascular adaptations to exercise training. Intriguingly, ERRα may attempt to compensate for the menopause-related loss of ERE activation, as ERRα protein content increased significantly in postmenopausal women following a period of exercise training, an effect that was not observed in premenopausal women (Nyberg et al., 2014). Importantly, in postmenopausal women, there may be an optimal window for contraction-mediated training adaptations, as ERRα protein content is negatively correlated with years after menopause in sedentary women, suggesting that the opportunity for accruing beneficial cardiovascular adaptations to exercise training may decrease with increasing time after menopause (Gliemann and Hellsten, 2019). Second, atherosclerotic plaque development begins when endothelial cells are repeatedly exposed to a suboptimal environment (e.g., poor lipid profile, hypertension, free radicals). The progression of atherosclerosis begins with functional impairments to resistance vessels and moves to structural alterations, such as arterial stiffening (Francis and Pierce, 2011). It is more difficult to reverse long-lasting structural changes to the vasculature as

opposed to circulating biomarkers that trigger functional impairments to the vessel (Francis and Pierce, 2011). Third, aging and menopause are associated with increased reactive oxygen species production and concomitant impairments to the endogenous antioxidant defense system, resulting in increased oxidative stress (van Beek et al., 2016). Exercise acutely increases skeletal muscle reactive oxygen species production, an effect that is critical for exercise training adaptations. In young, healthy individuals, the endogenous antioxidant systems can combat this acute rise in reactive oxygen species. However, with aging and menopause it is possible that ROS formation exceeds the capacity of the antioxidant systems, thereby blunting the potential for beneficial adaptations to exercise training.

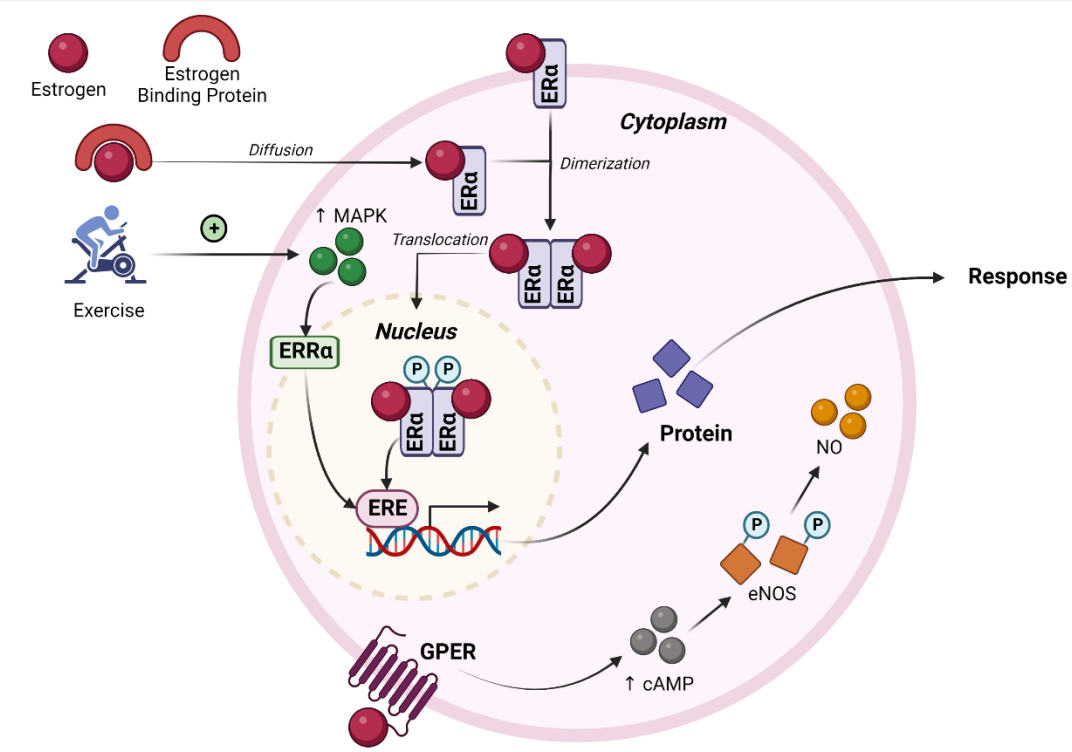

Figure 2-8: Estrogen-related receptor α (ERRα) and exercise.

ERRα can be activated by exercise and has been shown to regulate many of the same signalling pathways as endogenous estrogen. MAPK; mitogen-activated protein kinase, ERα; estrogen receptor alpha, ERE; estrogen response element, GPER; G protein-coupled estrogen receptor, cAMP; cyclic adenosine monophosphate, eNOS; endothelial nitric oxide synthase, NO; nitric oxide (Wickham, *unpublished*).

2.9 Menopause, Phytoestrogens, and Vascular Health

Recently, a growing interest in the role of phytoestrogens as a natural means to promote vascular health has emerged. Phytoestrogens are non-steroidal compounds derived from plants or plant precursors, and belong to one of three classes: isoflavones, lignans, or coumestans (Bedell et al., 2014). A particular focus has been placed on isoflavones, such as soybeans and red clover, due to their potent estrogenic activity, relative to other phytoestrogens (Vitale et al., 2013).

2.9.1 Isoflavones and Vascular Health

Following consumption, isoflavones are enzymatically converted to aglycones (e.g., genistein and daidzein) in the gut and released into the circulation (Bedell et al., 2014). The chemical composition of aglycones contains a phenolic ring (Rahman et al., 2022), which can mimic the roles of endogenous estrogens by binding to both estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) (Pfitscher et al., 2008; Pilšáková et al., 2010). Compared to endogenous estrogens, phytoestrogens have weak estrogenic activity (Viggiani et al., 2019) and although endogenous estrogens binds with equal affinity to ERα and ERβ (Couse et al., 1997), phytoestrogens appear to have a higher affinity for ERβ (Pfitscher et al., 2008). Previous research has shown that women ingesting large quantities of phytoestrogens (e.g., through diet or supplementation) are less likely to develop cardiovascular disease as well as breast and uterine cancer (Horn-Ross et al., 2003; Sathyapalan et al., 2018). Long-term (≥6 weeks) soy isoflavone supplementation studies have shown that this may be attributed to greater NO bioavailability (Squadrito et al., 2002) and prostacyclin release (García-Martínez et al., 2003) as well as reduced endothelin-1 levels (Squadrito et al., 2002) in postmenopausal women. Moreover, long-term soy isoflavone supplementation can improve markers of vascular inflammation in postmenopausal women (Colacurci et al., 2005; Hall et al., 2005; Lebon et al., 2014). Importantly, these positive changes in vascular biomarkers with soy isoflavone supplementation may contribute to an improvement in vascular function (S.-H. Li et al., 2010).

2.9.2 Fermented Red Clover Extract and Vascular Health

Red clover (trifolium pratense), a plant known for its high phytoestrogen content, is a novel therapeutic intervention for postmenopausal women. This is attributed to its high isoflavone content, particularly biochanin A and formononetin (metabolites of genistein and daidzein) (Lemežienė et al., 2015). Though limited, previous research has shown that 4 to 5 weeks of supplementation with red clover extract can confer positive effects on vascular function in postmenopausal women (Howes et al., 2003; Nestel et al., 1999). However, the findings regarding blood markers of cardiovascular health are currently unclear. Some evidence suggests improvements in antioxidant and vasorelaxant properties (Kim et al., 2020) as well as atherogenic adhesion molecules (Simoncini et al., 2008), while other studies show no effect of red clover extract on inflammation (Thorup et al., 2015) or coagulation (Mainini et al., 2013). Notably, recent advances in food science and nutraceutical biochemistry have used the fermentation process, converting glycosides to aglycones (Thorup et al., 2015), to significantly improve the bioavailability and therapeutic potential of red clover isoflavones (Ting et al., 2014). Therefore, although the research is in its infancy, fermented red clover extract (RC) may be a safe, easily accessible, and highly efficacious therapeutic intervention to promote vascular health in postmenopausal women.

2.10 Gaps in the Literature

Over the last 10 years, there has been a growing emphasis on the underrepresentation of women in human physiology research (Costello et al., 2014; Ribeiro et al., 2022; Smith et al., 2022). Although leaps and bounds have been made to encourage female-specific

inclusion, this work has tended to focus on the inclusion of young, healthy women. Ribeiro et al. (2022) published a review article summarizing the findings regarding the benefits of physical exercise in postmenopausal women, where only five research articles were included between 2014 and 2018, highlighting the scarcity of this research. Moreover, a recent study reported that women are underrepresented in human cardiovascular clinical trials aimed at verifying the efficacy of common therapeutics. Specifically, women comprised only 24% of participants in ischemic heart disease and heart failure trials, which are the most common cardiovascular conditions affecting women (Pilote and Raparelli, 2018). Another study highlighted that older women are often excluded from clinical trials studying ischemic heart disease, even though the disease is more prevalent in older populations (Bourgeois et al., 2017). Together, major gaps clearly remain in our fundamental understanding of the physiological consequences of menopause as they relate to drug interventions and alternative therapies, such as exercise and phytoestrogen supplementation (Figure 2-9).

This dissertation aims to help bridge those gaps by contributing novel and comprehensive insights into the impact of exercise training and phytoestrogen supplementation on vascular health in postmenopausal women. The first project explores the synergy between exercise training and cardiovascular medications. The review and second project investigate the efficacy of the initiation of exercise training relative to the time after menopause. Finally, the third project investigates the short-term effects of a novel phytoestrogen supplement on vascular health outcomes.

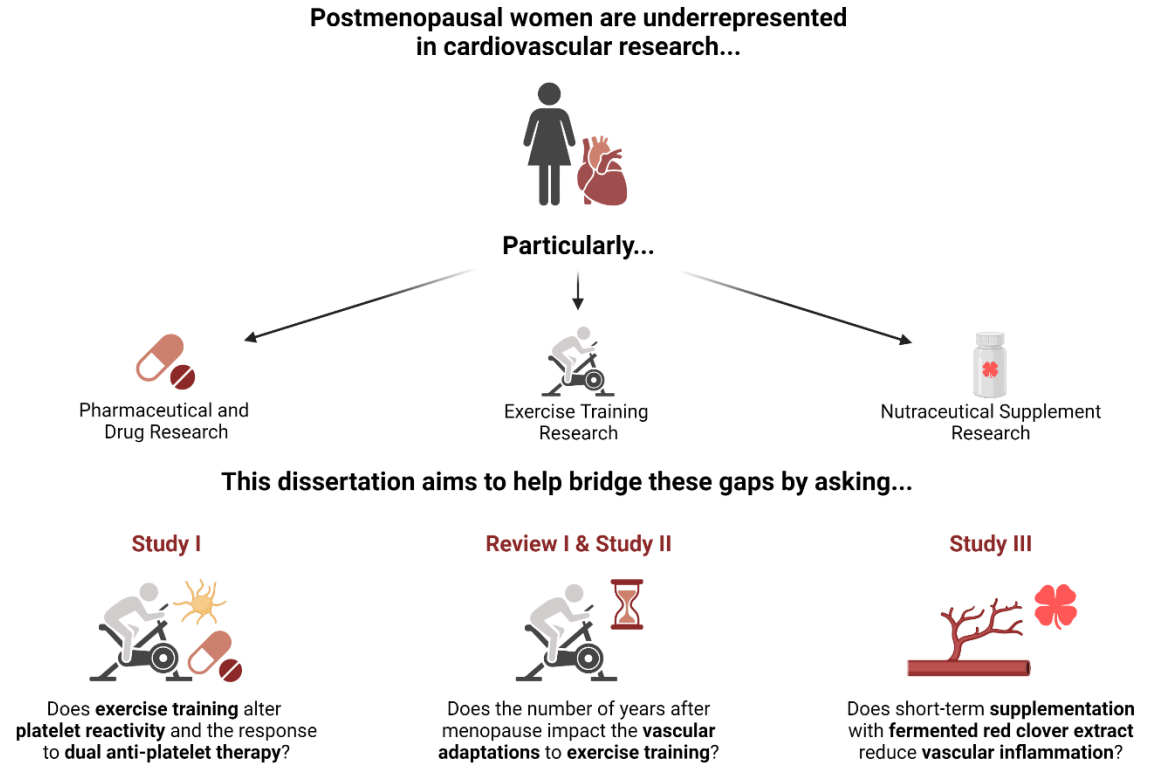

Figure 2-9: Overview of the knowledge gap that the dissertation aims to address (Wickham, unpublished*).*

2.11 References

Adair, J.G., 1984. The Hawthorne effect: A reconsideration of the methodological artifact. Journal of Applied Psychology 69, 334–345. https://doi.org/10.1037/0021-9010.69.2.334

Alfarisi, H.A.H., Mohamed, Z.B.H., Ibrahim, M.B., 2020. Basic pathogenic mechanisms of atherosclerosis. Egyptian Journal of Basic and Applied Sciences 7, 116–125. https://doi.org/10.1080/2314808X.2020.1769913

Alkarithi, G., Duval, C., Shi, Y., Macrae, F.L., Ariëns, R.A.S., 2021. Thrombus Structural Composition in Cardiovascular Disease. Arteriosclerosis, Thrombosis, and Vascular Biology 41, 2370–2383. https://doi.org/10.1161/ATVBAHA.120.315754

Badeau, M., Adlercreutz, H., Kaihovaara, P., Tikkanen, M.J., 2005. Estrogen A-ring structure and antioxidative effect on lipoproteins. The Journal of Steroid Biochemistry and Molecular Biology 96, 271–278. https://doi.org/10.1016/j.jsbmb.2005.04.034

Balsano, F., Rizzon, P., Violi, F., Scrutinio, D., Cimminiello, C., Aguglia, F., Pasotti, C., Rudelli, G., 1990. Antiplatelet treatment with ticlopidine in unstable angina. A controlled multicenter clinical trial. The Studio della Ticlopidina nell'Angina Instabile Group. Circulation 82, 17–26. https://doi.org/10.1161/01.cir.82.1.17

Barton, M., Meyer, M.R., 2009. Postmenopausal hypertension: mechanisms and therapy. Hypertension 54, 11–18. https://doi.org/10.1161/HYPERTENSIONAHA.108.120022

Batty, P., Smith, J.G., 2010. Haemostasis. Surgery (Oxford), Emergency Surgery 28, 530–535. https://doi.org/10.1016/j.mpsur.2010.08.008

Bedell, S., Nachtigall, M., Naftolin, F., 2014. The pros and cons of plant estrogens for menopause. The Journal of Steroid Biochemistry and Molecular Biology 139, 225–236. https://doi.org/10.1016/j.jsbmb.2012.12.004

Bejma, J., Ji, L.L., 1999. Aging and acute exercise enhance free radical generation in rat skeletal muscle. J Appl Physiol (1985) 87, 465–470. https://doi.org/10.1152/jappl.1999.87.1.465

Bergstrom, J., 1975. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scand J Clin Lab Invest 35, 609–616.

Bertrand, M.E., Rupprecht, H.J., Urban, P., Gershlick, A.H., CLASSICS Investigators, 2000. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting : the clopidogrel aspirin stent international cooperative study (CLASSICS). Circulation 102, 624–629. https://doi.org/10.1161/01.cir.102.6.624

Bittner, V., 2009. Menopause, Age, and Cardiovascular Risk. Journal of the American College of Cardiology 54, 2374–2375. https://doi.org/10.1016/j.jacc.2009.10.008

Bossavy, J.-P., Thalamas, C., Sagnard, L., Barret, A., Sakariassen, K., Boneu, B., Cadroy, Y., 1998. A Double-Blind Randomized Comparison of Combined Aspirin and Ticlopidine Therapy Versus Aspirin or Ticlopidine Alone on Experimental Arterial

Thrombogenesis in Humans. Blood 92, 1518–1525. https://doi.org/10.1182/blood.V92.5.1518
Bouchard, C., An, P., Rice, T., Skinner, J.S., Wilmore, J.H., Gagnon, J., Pérusse, L., Leon, A.S., Rao, D.C., 1999. Familial aggregation ofV˙o 2 max response to exercise training: results from the HERITAGE Family Study. Journal of Applied Physiology 87, 1003–1008. https://doi.org/10.1152/jappl.1999.87.3.1003
Bourgeois, F.T., Orenstein, L., Ballakur, S., Mandl, K.D., Ioannidis, J.P.A., 2017. Exclusion of Elderly People from Randomized Clinical Trials of Drugs for Ischemic Heart Disease. J Am Geriatr Soc 65, 2354–2361. https://doi.org/10.1111/jgs.14833
Brass, L.F., 2003. Thrombin and Platelet Activation. Chest 124, 18S-25S. https://doi.org/10.1378/chest.124.3_suppl.18S
Breet, N.J., Sluman, M.A., van Berkel, M.A.J.P.J., van Werkum, J.W., Bouman, H.J., Harmsze, A.M., Kelder, J.C., Zijlstra, F., Hackeng, C.M., ten Berg, J.M., 2011. Effect of gender difference on platelet reactivity. Neth Heart J 19, 451–457. https://doi.org/10.1007/s12471-011-0189-y
Briasoulis, A., Telila, T., Palla, M., Siasos, G., Tousoulis, D., 2016. P2Y12 Receptor Antagonists: Which One to Choose? A Systematic Review and Meta-Analysis. Curr Pharm Des 22, 4568–4576. https://doi.org/10.2174/1381612822666160608114424
Brown, R.G., Davidson, L.A.G., Mckeown, T., Whitfield, A.G.W., 1957. CORONARY-ARTERY DISEASE: INFLUENCES AFFECTING ITS INCIDENCE IN MALES IN THE SEVENTH DECADE. The Lancet, Originally published as Volume 2, Issue 7005 270, 1073–1077. https://doi.org/10.1016/S0140-6736(57)90113-7
Brownley, K.A., Hinderliter, A.L., West, S.G., Grewen, K.M., Steege, J.F., Girdler, S.S., Light, K.C., 2004. Cardiovascular effects of 6 months of hormone replacement therapy versus placebo: differences associated with years since menopause. American Journal of Obstetrics and Gynecology 190, 1052–1058. https://doi.org/10.1016/j.ajog.2003.09.045
Brzozowska, M., Lewiński, A., 2020. Changes of androgens levels in menopausal women. Prz Menopauzalny 19, 151–154. https://doi.org/10.5114/pm.2020.101941
Burger, H.G., 2002. Androgen production in women. Fertil Steril 77 Suppl 4, S3-5. https://doi.org/10.1016/s0015-0282(02)02985-0
Cauley, J.A., Robbins, J., Chen, Z., Cummings, S.R., Jackson, R.D., LaCroix, A.Z., LeBoff, M., Lewis, C.E., McGowan, J., Neuner, J., Pettinger, M., Stefanick, M.L., Wactawski-Wende, J., Watts, N.B., for the Women's Health Initiative Investigators, 2003. Effects of Estrogen Plus Progestin on Risk of Fracture and Bone Mineral DensityThe Women's Health Initiative Randomized Trial. JAMA 290, 1729–1738. https://doi.org/10.1001/jama.290.13.1729
Celermajer, D.S., Sorensen, K.E., Spiegelhalter, D.J., Georgakopoulos, D., Robinson, J., Deanfield, J.E., 1994. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol 24, 471–476. https://doi.org/10.1016/0735-1097(94)90305-0
Chan, M.V., Knowles, R.B.M., Lundberg, M.H., Tucker, A.T., Mohamed, N.A., Kirkby, N.S., Armstrong, P.C.J., Mitchell, J.A., Warner, T.D., 2016. P2Y12 receptor blockade

synergizes strongly with nitric oxide and prostacyclin to inhibit platelet activation. Br J Clin Pharmacol 81, 621–633. https://doi.org/10.1111/bcp.12826

Chapin, J.C., Hajjar, K.A., 2015. Fibrinolysis and the control of blood coagulation. Blood Rev 29, 17–24. https://doi.org/10.1016/j.blre.2014.09.003

Chlebowski, R.T., Wactawski-Wende, J., Ritenbaugh, C., Hubbell, F.A., Ascensao, J., Rodabough, R.J., Rosenberg, C.A., Taylor, V.M., Harris, R., Chen, C., Adams-Campbell, L.L., White, E., 2004. Estrogen plus Progestin and Colorectal Cancer in Postmenopausal Women. New England Journal of Medicine 350, 991–1004. https://doi.org/10.1056/NEJMoa032071

Cho, L., Mukherjee, D., 2005. Hormone replacement therapy and secondary cardiovascular prevention: a meta-analysis of randomized trials. Cardiology 104, 143–147. https://doi.org/10.1159/000087635

Chrzanowski-Smith, O.J., Piatrikova, E., Betts, J.A., Williams, S., Gonzalez, J.T., 2020. Variability in exercise physiology: Can capturing intra-individual variation help better understand true inter-individual responses? European Journal of Sport Science 20, 452–460. https://doi.org/10.1080/17461391.2019.1655100

Ciana, P., Raviscioni, M., Mussi, P., Vegeto, E., Que, I., Parker, M.G., Lowik, C., Maggi, A., 2003. In vivo imaging of transcriptionally active estrogen receptors. Nat Med 9, 82–86. https://doi.org/10.1038/nm809

Colacurci, N., Chiàntera, A., Fornaro, F., de Novellis, V., Manzella, D., Arciello, A., Chiàntera, V., Improta, L., Paolisso, G., 2005. Effects of soy isoflavones on endothelial function in healthy postmenopausal women. Menopause 12, 299–307. https://doi.org/10.1097/01.GME.0000147017.23173.5B

Collet, J.-P., Thiele, H., Barbato, E., Barthélémy, O., Bauersachs, J., Bhatt, D.L., Dendale, P., Dorobantu, M., Edvardsen, T., Folliguet, T., Gale, C.P., Gilard, M., Jobs, A., Jüni, P., Lambrinou, E., Lewis, B.S., Mehilli, J., Meliga, E., Merkely, B., Mueller, C., Roffi, M., Rutten, F.H., Sibbing, D., Siontis, G.C.M., ESC Scientific Document Group, Kastrati, A., Mamas, M.A., Aboyans, V., Angiolillo, D.J., Bueno, H., Bugiardini, R., Byrne, R.A., Castelletti, S., Chieffo, A., Cornelissen, V., Crea, F., Delgado, V., Drexel, H., Gierlotka, M., Halvorsen, S., Haugaa, K.H., Jankowska, E.A., Katus, H.A., Kinnaird, T., Kluin, J., Kunadian, V., Landmesser, U., Leclercq, C., Lettino, M., Meinila, L., Mylotte, D., Ndrepepa, G., Omerovic, E., Pedretti, R.F.E., Petersen, S.E., Petronio, A.S., Pontone, G., Popescu, B.A., Potpara, T., Ray, K.K., Luciano, F., Richter, D.J., Shlyakhto, E., Simpson, I.A., Sousa-Uva, M., Storey, R.F., Touyz, R.M., Valgimigli, M., Vranckx, P., Yeh, R.W., Barbato, E., Barthélémy, O., Bauersachs, J., Bhatt, D.L., Dendale, P., Dorobantu, M., Edvardsen, T., Folliguet, T., Gale, C.P., Gilard, M., Jobs, A., Jüni, P., Lambrinou, E., Lewis, B.S., Mehilli, J., Meliga, E., Merkely, B., Mueller, C., Roffi, M., Rutten, F.H., Sibbing, D., Siontis, G.C.M., 2021. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. European Heart Journal 42, 1289–1367. https://doi.org/10.1093/eurheartj/ehaa575

Costello, J.T., Bieuzen, F., Bleakley, C.M., 2014. Where are all the female participants in Sports and Exercise Medicine research? European Journal of Sport Science 14, 847–851. https://doi.org/10.1080/17461391.2014.911354
Couse, J.F., Lindzey, J., Grandien, K., Gustafsson, J.A., Korach, K.S., 1997. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 138, 4613–4621. https://doi.org/10.1210/endo.138.11.5496
Craige, S.M., Kröller-Schön, S., Li, C., Kant, S., Cai, S., Chen, K., Contractor, M.M., Pei, Y., Schulz, E., Keaney, J.F., 2016. PGC-1α dictates endothelial function through regulation of eNOS expression. Sci Rep 6, 38210. https://doi.org/10.1038/srep38210
Curtis, D.J., Brown, M.R., Hawkins, K., Evans, P.A., Lawrence, M.J., Rees, P., Williams, P.R., 2011. Rheometrical and molecular dynamics simulation studies of incipient clot formation in fibrin-thrombin gels: An activation limited aggregation approach. Journal of Non-Newtonian Fluid Mechanics, Papers Presented at the University of Wales Institute of Non-Newtonian Fluid Mechanics Meeting on Rheometry, 29-31 March 2010, Lake Vyrnwy, Wales 166, 932–938. https://doi.org/10.1016/j.jnnfm.2011.04.016
Curtis, D.J., Williams, P.R., Badiei, N., Campbell, A.I., Hawkins, K., Evans, P.A., Brown, M.R., 2013. A study of microstructural templating in fibrin–thrombin gel networks by spectral and viscoelastic analysis. Soft Matter 9, 4883–4889. https://doi.org/10.1039/C3SM50263E
Danforth, D.R., Arbogast, L.K., Mroueh, J., Kim, M.H., Kennard, E.A., Seifer, D.B., Friedman, C.I., 1998. Dimeric inhibin: a direct marker of ovarian aging. Fertil Steril 70, 119–123. https://doi.org/10.1016/s0015-0282(98)00127-7
Dastjerdi, M.S., Emami, T., Najafian, A., Amini, M., 2006. Mean platelet volume measurement, EDTA or citrate? Hematology 11, 317–319. https://doi.org/10.1080/10245330600954163
Davie, E.W., Ratnoff, O.D., 1964. Waterfall Sequence for Intrinsic Blood Clotting. Science 145, 1310–1312. https://doi.org/10.1126/science.145.3638.1310
Davies, N.A., Llwyd, O., Brugniaux, J.V., Davies, G.R., Marley, C.J., Hodson, D., Lawrence, M.J., D'Silva, L.A., Morris, R.H.K., Hawkins, K., Williams, P.R., Bailey, D.M., Evans, P.A., 2016. Effects of exercise intensity on clot microstructure and mechanical properties in healthy individuals. Thrombosis Research 143, 130–136. https://doi.org/10.1016/j.thromres.2016.05.018
DeSouza, C.A., Shapiro, L.F., Clevenger, C.M., Dinenno, F.A., Monahan, K.D., Tanaka, H., Seals, D.R., 2000. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 102, 1351–1357. https://doi.org/10.1161/01.cir.102.12.1351
Dhalla, N.S., Temsah, R.M., Netticadan, T., 2000. Role of oxidative stress in cardiovascular diseases. J Hypertens 18, 655–673. https://doi.org/10.1097/00004872-200018060-00002

Dobesh, P.P., Oestreich, J.H., 2014. Ticagrelor: Pharmacokinetics, Pharmacodynamics, Clinical Efficacy, and Safety. Pharmacotherapy 34, 1077–1090. https://doi.org/10.1002/phar.1477

Dorsam, R.T., Kunapuli, S.P., 2004. Central role of the $P2Y_{12}$ receptor in platelet activation. J Clin Invest 113, 340–345. https://doi.org/10.1172/JCI20986

Egelund, J., Jørgensen, P.G., Mandrup, C.M., Fritz-Hansen, T., Stallknecht, B., Bangsbo, J., Nyberg, M., Hellsten, Y., 2017. Cardiac Adaptations to High-Intensity Aerobic Training in Premenopausal and Recent Postmenopausal Women: The Copenhagen Women Study. J Am Heart Assoc 6, e005469. https://doi.org/10.1161/JAHA.117.005469

El Khoudary, S.R., Aggarwal, B., Beckie, T.M., Hodis, H.N., Johnson, A.E., Langer, R.D., Limacher, M.C., Manson, J.E., Stefanick, M.L., Allison, M.A., null, null, 2020. Menopause Transition and Cardiovascular Disease Risk: Implications for Timing of Early Prevention: A Scientific Statement From the American Heart Association. Circulation 142, e506–e532. https://doi.org/10.1161/CIR.0000000000000912

Elmariah, S., Mauri, L., Doros, G., Galper, B.Z., O'Neill, K.E., Steg, P.G., Kereiakes, D.J., Yeh, R.W., 2015. Extended duration dual antiplatelet therapy and mortality: a systematic review and meta-analysis. The Lancet 385, 792–798. https://doi.org/10.1016/S0140-6736(14)62052-3

Engbers, M.J., Van Hylckama Vlieg, A., Rosendaal, F.R., 2010. Venous thrombosis in the elderly: incidence, risk factors and risk groups. Journal of Thrombosis and Haemostasis 8, 2105–2112. https://doi.org/10.1111/j.1538-7836.2010.03986.x

Finkelstein, J.S., Brockwell, S.E., Mehta, V., Greendale, G.A., Sowers, M.R., Ettinger, B., Lo, J.C., Johnston, J.M., Cauley, J.A., Danielson, M.E., Neer, R.M., 2008. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J Clin Endocrinol Metab 93, 861–868. https://doi.org/10.1210/jc.2007-1876

FitzGerald, G.A., Oates, J.A., Hawiger, J., Maas, R.L., Roberts, L.J., Lawson, J.A., Brash, A.R., 1983. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest 71, 676–688. https://doi.org/10.1172/JCI110814

Fleming, I., 2010. Molecular mechanisms underlying the activation of eNOS. Pflugers Arch 459, 793–806. https://doi.org/10.1007/s00424-009-0767-7

Fogle, R.H., Stanczyk, F.Z., Zhang, X., Paulson, R.J., 2007. Ovarian androgen production in postmenopausal women. J Clin Endocrinol Metab 92, 3040–3043. https://doi.org/10.1210/jc.2007-0581

Förstermann, U., Münzel, T., 2006. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113, 1708–1714. https://doi.org/10.1161/CIRCULATIONAHA.105.602532

Fournier, A., Mesrine, S., Dossus, L., Boutron-Ruault, M.-C., Clavel-Chapelon, F., Chabbert-Buffet, N., 2014. Risk of breast cancer after stopping menopausal hormone therapy in the E3N cohort. Breast Cancer Res Treat 145, 535–543. https://doi.org/10.1007/s10549-014-2934-6

Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri, M., Salvioli, S., 2018. The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. Frontiers in Medicine 5.

Francis, A.A., Pierce, G.N., 2011. An integrated approach for the mechanisms responsible for atherosclerotic plaque regression. Exp Clin Cardiol 16, 77–86.

Fredette, N.C., Meyer, M.R., Prossnitz, E.R., 2018. Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. J Steroid Biochem Mol Biol 176, 65–72. https://doi.org/10.1016/j.jsbmb.2017.05.006

Fuentes, N., Silveyra, P., 2019. Estrogen receptor signaling mechanisms. Advances in protein chemistry and structural biology 116, 135. https://doi.org/10.1016/bs.apcsb.2019.01.001

Fuller, R., Chavez, B., 2012. Ticagrelor (Brilinta), an Antiplatelet Drug for Acute Coronary Syndrome. P T 37, 562–568.

Gale, A.J., 2011. Current Understanding of Hemostasis. Toxicol Pathol 39, 273–280. https://doi.org/10.1177/0192623310389474

García-Martínez, M.C., Hermenegildo, C., Tarín, J.J., Cano, A., 2003. Phytoestrogens increase the capacity of serum to stimulate prostacyclin release in human endothelial cells. Acta Obstet Gynecol Scand 82, 705–710. https://doi.org/10.1080/j.1600-0412.2003.00178.x

Gent, M., Blakely, J.A., Easton, J.D., Ellis, D.J., Hachinski, V.C., Harbison, J.W., Panak, E., Roberts, R.S., Sicurella, J., Turpie, A.G., 1989. The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. Lancet 1, 1215–1220. https://doi.org/10.1016/s0140-6736(89)92327-1

Gliemann, L., Hellsten, Y., 2019. The exercise timing hypothesis: can exercise training compensate for the reduction in blood vessel function after menopause if timed right? The Journal of Physiology 597, 4915–4925. https://doi.org/10.1113/JP277056

Gliemann, L., Rytter, N., Tamariz-Ellemann, A., Egelund, J., Brandt, N., Carter, H.H., Hellsten, Y., 2020. Lifelong Physical Activity Determines Vascular Function in Late Postmenopausal Women. Med Sci Sports Exerc 52, 627–636. https://doi.org/10.1249/MSS.0000000000002180

Goldman, J.A., 2004. The Women's Health Initiative 2004 - Review and Critique. MedGenMed 6, 65.

Government of Canada, 2021. Canadian Health Measures Survey: Activity monitor data, 2018-2019 [WWW Document]. URL https://www150.statcan.gc.ca/n1/daily-quotidien/210901/dq210901c-eng.htm (accessed 10.12.22).

Grady, D., Rubin, S.M., Petitti, D.B., Fox, C.S., Black, D., Ettinger, B., Ernster, V.L., Cummings, S.R., 1992. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 117, 1016–1037. https://doi.org/10.7326/0003-4819-117-12-1016

Green, D.J., Maiorana, A., O'Driscoll, G., Taylor, R., 2004. Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol 561, 1–25. https://doi.org/10.1113/jphysiol.2004.068197

Greendale, G.A., Reboussin, B.A., Hogan, P., Barnabei, V.M., Shumaker, S., Johnson, S., Barrett-Connor, E., 1998. Symptom relief and side effects of postmenopausal hormones: results from the postmenopausal estrogen/progestin interventions trial. Obstetrics & Gynecology 92, 982–988. https://doi.org/10.1016/S0029-7844(98)00305-6

Gregorini, L., Marco, J., 1997. Ticlopidine and aspirin interactions. Heart 77, 11–12. https://doi.org/10.1136/hrt.77.1.11

Grindler, N.M., Santoro, N.F., 2015. Menopause and exercise. Menopause 22, 1351–1358. https://doi.org/10.1097/GME.0000000000000536

Grodstein, F., Stampfer, M.J., Colditz, G.A., Willett, W.C., Manson, J.E., Joffe, M., Rosner, B., Fuchs, C., Hankinson, S.E., Hunter, D.J., Hennekens, C.H., Speizer, F.E., 1997. Postmenopausal Hormone Therapy and Mortality. New England Journal of Medicine 336, 1769–1776. https://doi.org/10.1056/NEJM199706193362501

Hackley, B., Rousseau, M.E., 2004. Managing menopausal symptoms after the women's health initiative. Journal of Midwifery & Women's Health 49, 87–95. https://doi.org/10.1016/j.jmwh.2003.12.005

Hall, W.L., Vafeiadou, K., Hallund, J., Bügel, S., Koebnick, C., Reimann, M., Ferrari, M., Branca, F., Talbot, D., Dadd, T., Nilsson, M., Dahlman-Wright, K., Gustafsson, J.-A., Minihane, A.-M., Williams, C.M., 2005. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. Am J Clin Nutr 82, 1260–1268; quiz 1365–1366. https://doi.org/10.1093/ajcn/82.6.1260

Hamilos, M., Petousis, S., Parthenakis, F., 2018. Interaction between platelets and endothelium: from pathophysiology to new therapeutic options. Cardiovasc Diagn Ther 8, 568–580. https://doi.org/10.21037/cdt.2018.07.01

Hamilton, K.J., Hewitt, S.C., Arao, Y., Korach, K.S., 2017. Estrogen Hormone Biology. Curr Top Dev Biol 125, 109–146. https://doi.org/10.1016/bs.ctdb.2016.12.005

Hanby, H.A., Bao, J., Noh, J.-Y., Jarocha, D., Poncz, M., Weiss, M.J., Marks, M.S., 2017. Platelet dense granules begin to selectively accumulate mepacrine during proplatelet formation. Blood Advances 1, 1478–1490. https://doi.org/10.1182/bloodadvances.2017006726

Haney, A., Wild, R., 2007. Options for hormone therapy in women who have had a hysterectomy. Menopause (New York, N.Y.) 14. https://doi.org/10.1097/gme.0b013e31804154d5

Harlow, S.D., Mitchell, E.S., Crawford, S., Nan, B., Little, R., Taffe, J., ReSTAGE Collaboration, 2008. The ReSTAGE Collaboration: defining optimal bleeding criteria for onset of early menopausal transition. Fertil Steril 89, 129–140. https://doi.org/10.1016/j.fertnstert.2007.02.015

Harper-Harrison, G., Shanahan, M.M., 2022. Hormone Replacement Therapy, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Harridge, S.D.R., Lazarus, N.R., 2017. Physical Activity, Aging, and Physiological Function. Physiology (Bethesda) 32, 152–161. https://doi.org/10.1152/physiol.00029.2016

Harris, G.W., 1948. Electrical stimulation of the hypothalamus and the mechanism of neural control of the adenohypophysis. J Physiol 107, 418–429. https://doi.org/10.1113/jphysiol.1948.sp004286

Hass, W.K., Easton, J.D., Adams, H.P., Pryse-Phillips, W., Molony, B.A., Anderson, S., Kamm, B., 1989. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. Ticlopidine Aspirin Stroke Study Group. N Engl J Med 321, 501–507. https://doi.org/10.1056/NEJM198908243210804

Hawley, J.A., Burke, L.M., Phillips, S.M., Spriet, L.L., 2011. Nutritional modulation of training-induced skeletal muscle adaptations. J Appl Physiol (1985) 110, 834–845. https://doi.org/10.1152/japplphysiol.00949.2010

He, F., Li, J., Liu, Z., Chuang, C.-C., Yang, W., Zuo, L., 2016. Redox Mechanism of Reactive Oxygen Species in Exercise. Front Physiol 7, 486. https://doi.org/10.3389/fphys.2016.00486

Hecker, M., Ullrich, V., 1989. On the mechanism of prostacyclin and thromboxane A2 biosynthesis. J Biol Chem 264, 141–150.

Henderson, B.E., Paganini-Hill, A., Ross, R.K., 1991. Decreased Mortality in Users of Estrogen Replacement Therapy. Archives of Internal Medicine 151, 75–78. https://doi.org/10.1001/archinte.1991.00400010095012

Hiller, E., 2007. Basic Principles of Hemostasis, in: Munker, R., Hiller, E., Glass, J., Paquette, R. (Eds.), Modern Hematology: Biology and Clinical Management, Contemporary Hematology. Humana Press, Totowa, NJ, pp. 327–345. https://doi.org/10.1007/978-1-59745-149-9_19

Hinchliff, S., 2019. Menopause, in: Gu, D., Dupre, M.E. (Eds.), Encyclopedia of Gerontology and Population Aging. Springer International Publishing, Cham, pp. 1–5. https://doi.org/10.1007/978-3-319-69892-2_161-1

Hodis, H.N., Mack, W.J., Henderson, V.W., Shoupe, D., Budoff, M.J., Hwang-Levine, J., Li, Y., Feng, M., Dustin, L., Kono, N., Stanczyk, F.Z., Selzer, R.H., Azen, S.P., 2016. Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol. N Engl J Med 374, 1221–1231. https://doi.org/10.1056/NEJMoa1505241

Hoffman, B.B., Michel, T., Brenneman, T.B., Lefkowitz, R.J., 1982. Interactions of agonists with platelet alpha 2-adrenergic receptors. Endocrinology 110, 926–932. https://doi.org/10.1210/endo-110-3-926

Hoier, B., Nordsborg, N., Andersen, S., Jensen, L., Nybo, L., Bangsbo, J., Hellsten, Y., 2012. Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. The Journal of Physiology 590, 595–606. https://doi.org/10.1113/jphysiol.2011.216135

Hoier, B., Olsen, L.N., Leinum, M., Jørgensen, T.S., Carter, H.H., Hellsten, Y., Bangsbo, J., 2021. Aerobic High-Intensity Exercise Training Improves Cardiovascular Health in Older Post-menopausal Women. Front Aging 2, 667519. https://doi.org/10.3389/fragi.2021.667519

Holder, S.M., Brislane, Á., Dawson, E.A., Hopkins, N.D., Hopman, M.T.E., Cable, N.T., Jones, H., Schreuder, T.H.A., Sprung, V.S., Naylor, L., Maiorana, A., Thompson, A., Thijssen, D.H.J., Green, D.J., 2019. Relationship Between Endothelial Function

and the Eliciting Shear Stress Stimulus in Women: Changes Across the Lifespan Differ to Men. J Am Heart Assoc 8, e010994. https://doi.org/10.1161/JAHA.118.010994

Horn-Ross, P.L., John, E.M., Canchola, A.J., Stewart, S.L., Lee, M.M., 2003. Phytoestrogen intake and endometrial cancer risk. J Natl Cancer Inst 95, 1158–1164. https://doi.org/10.1093/jnci/djg015

Horstman, A.M., Dillon, E.L., Urban, R.J., Sheffield-Moore, M., 2012. The Role of Androgens and Estrogens on Healthy Aging and Longevity. J Gerontol A Biol Sci Med Sci 67, 1140–1152. https://doi.org/10.1093/gerona/gls068

Howes, J.B., Tran, D., Brillante, D., Howes, L.G., 2003. Effects of dietary supplementation with isoflavones from red clover on ambulatory blood pressure and endothelial function in postmenopausal type 2 diabetes. Diabetes Obes Metab 5, 325–332. https://doi.org/10.1046/j.1463-1326.2003.00282.x

Hsia, J., Langer, R.D., Manson, J.E., Kuller, L., Johnson, K.C., Hendrix, S.L., Pettinger, M., Heckbert, S.R., Greep, N., Crawford, S., Eaton, C.B., Kostis, J.B., Caralis, P., Prentice, R., Women's Health Initiative Investigators, 2006. Conjugated equine estrogens and coronary heart disease: the Women's Health Initiative. Arch Intern Med 166, 357–365. https://doi.org/10.1001/archinte.166.3.357

Huveneers, S., Daemen, M.J.A.P., Hordijk, P.L., 2015. Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. Circ Res 116, 895–908. https://doi.org/10.1161/CIRCRESAHA.116.305720

Hyde, E.T., Whitfield, G.P., Omura, J.D., Fulton, J.E., Carlson, S.A., 2021. Trends in Meeting the Physical Activity Guidelines: Muscle-Strengthening Alone and Combined With Aerobic Activity, United States, 1998–2018. Journal of Physical Activity and Health 18, S37–S44. https://doi.org/10.1123/jpah.2021-0077

Iqbal, J., Zaidi, M., 2009. Understanding Estrogen Action during Menopause. Endocrinology 150, 3443–3445. https://doi.org/10.1210/en.2009-0449

Izquierdo, M., Häkkinen, K., Antón, A., Garrues, M., Ibañez, J., Ruesta, M., Gorostiaga, E.M., 2001. Maximal strength and power, endurance performance, and serum hormones in middle-aged and elderly men. Med Sci Sports Exerc 33, 1577–1587. https://doi.org/10.1097/00005768-200109000-00022

Izzo, C., Vitillo, P., Di Pietro, P., Visco, V., Strianese, A., Virtuoso, N., Ciccarelli, M., Galasso, G., Carrizzo, A., Vecchione, C., 2021. The Role of Oxidative Stress in Cardiovascular Aging and Cardiovascular Diseases. Life (Basel) 11, 60. https://doi.org/10.3390/life11010060

Jin, J., Daniel, J.L., Kunapuli, S.P., 1998. Molecular basis for ADP-induced platelet activation. II. The P2Y1 receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. J Biol Chem 273, 2030–2034. https://doi.org/10.1074/jbc.273.4.2030

Jones, S.P., Bolli, R., 2006. The ubiquitous role of nitric oxide in cardioprotection. Journal of Molecular and Cellular Cardiology 40, 16–23. https://doi.org/10.1016/j.yjmcc.2005.09.011

Joo, J., Williamson, S.A., Vazquez, A.I., Fernandez, J.R., Bray, M.S., 2019. The influence of 15-week exercise training on dietary patterns among young adults. Int J Obes 43, 1681–1690. https://doi.org/10.1038/s41366-018-0299-3

Joswig, M., Hach-Wunderle, V., von Holst, T., Nawroth, P.P., 2000. Postmenopausal hormone replacement therapy and the vascular wall: epidemiology and clinical studies. Vasa 29, 243–251. https://doi.org/10.1024/0301-1526.29.4.243

Kalhan, M., Singhania, K., Choudhary, P., Verma, S., Kaushal, P., Singh, T., 2020. Prevalence of Menopausal Symptoms and its Effect on Quality of Life among Rural Middle Aged Women (40–60 Years) of Haryana, India. Int J Appl Basic Med Res 10, 183–188. https://doi.org/10.4103/ijabmr.IJABMR_428_19

Kerlikowske, K., Miglioretti, D.L., Ballard-Barbash, R., Weaver, D.L., Buist, D.S.M., Barlow, W.E., Cutter, G., Geller, B.M., Yankaskas, B., Taplin, S.H., Carney, P.A., 2003. Prognostic Characteristics of Breast Cancer Among Postmenopausal Hormone Users in a Screened Population. JCO 21, 4314–4321. https://doi.org/10.1200/JCO.2003.05.151

Kim, M.R., Kim, H.J., Yu, S.H., Lee, B.S., Jeon, S.Y., Lee, J.J., Lee, Y.C., 2020. Combination of Red Clover and Hops Extract Improved Menopause Symptoms in an Ovariectomized Rat Model. Evid Based Complement Alternat Med 2020, 7941391. https://doi.org/10.1155/2020/7941391

Kim, Y., Lee, E., 2019. The association between elderly people's sedentary behaviors and their health-related quality of life: focusing on comparing the young-old and the old-old. Health and Quality of Life Outcomes 17, 131. https://doi.org/10.1186/s12955-019-1191-0

Kirkby, N.S., Lundberg, M.H., Chan, M.V., Vojnovic, I., Solomon, A.B., Emerson, M., Mitchell, J.A., Warner, T.D., 2013. Blockade of the purinergic P2Y12 receptor greatly increases the platelet inhibitory actions of nitric oxide. Proc Natl Acad Sci U S A 110, 15782–15787. https://doi.org/10.1073/pnas.1218880110

Koenig, A., Buskiewicz, I., Huber, S.A., 2017. Age-Associated Changes in Estrogen Receptor Ratios Correlate with Increased Female Susceptibility to Coxsackievirus B3-Induced Myocarditis. Front Immunol 8, 1585. https://doi.org/10.3389/fimmu.2017.01585

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.A., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870. https://doi.org/10.1210/endo.138.3.4979

Lablanche, J.M., McFadden, E.P., Bonnet, J.L., Grollier, G., Danchin, N., Bedossa, M., Leclercq, C., Vahanian, A., Bauters, C., Van Belle, E., Bertrand, M.E., 1996. Combined antiplatelet therapy with ticlopidine and aspirin. A simplified approach to intracoronary stent management. Eur Heart J 17, 1373–1380. https://doi.org/10.1093/oxfordjournals.eurheartj.a015072

Laganière, J., Tremblay, G.B., Dufour, C.R., Giroux, S., Rousseau, F., Giguère, V., 2004. A polymorphic autoregulatory hormone response element in the human estrogen-related receptor alpha (ERRalpha) promoter dictates peroxisome proliferator-

activated receptor gamma coactivator-1alpha control of ERRalpha expression. J Biol Chem 279, 18504–18510. https://doi.org/10.1074/jbc.M313543200
Lawrence, M.J., Davies, G., Nyberg, M., Whitley, J., Evans, V., Williams, R., Hellsten, Y., Evans, P.A., 2018. The effect of tyramine infusion and exercise on blood flow, coagulation and clot microstructure in healthy individuals. Thrombosis Research 170, 32–37. https://doi.org/10.1016/j.thromres.2018.07.025
Lawrence, M.J., Kumar, S., Hawkins, K., Boden, S., Rutt, H., Mills, G., Sabra, A., Morris, R.H.K., Davidson, S.J., Badiei, N., Brown, M.R., Williams, P.R., Evans, P.A., 2014. A new structural biomarker that quantifies and predicts changes in clot strength and quality in a model of progressive haemodilution. Thrombosis Research 134, 488–494. https://doi.org/10.1016/j.thromres.2014.05.039
Lazarus, N.R., Harridge, S.D.R., 2017. Declining performance of master athletes: silhouettes of the trajectory of healthy human ageing? The Journal of Physiology 595, 2941–2948. https://doi.org/10.1113/JP272443
Le Blanc, J., Lordkipanidzé, M., 2019. Platelet Function in Aging. Front Cardiovasc Med 6, 109. https://doi.org/10.3389/fcvm.2019.00109
Lebon, J., Riesco, E., Tessier, D., Dionne, I.J., 2014. Additive effects of isoflavones and exercise training on inflammatory cytokines and body composition in overweight and obese postmenopausal women: a randomized controlled trial. Menopause 21, 869–875. https://doi.org/10.1097/GME.0000000000000177
Lecut, C., Schoolmeester, A., Kuijpers, M.J.E., Broers, J.L.V., van Zandvoort, M.A.M.J., Vanhoorelbeke, K., Deckmyn, H., Jandrot-Perrus, M., Heemskerk, J.W.M., 2004. Principal role of glycoprotein VI in alpha2beta1 and alphaIIbbeta3 activation during collagen-induced thrombus formation. Arterioscler Thromb Vasc Biol 24, 1727–1733. https://doi.org/10.1161/01.ATV.0000137974.85068.93
Lee, H.-L., Kim, J.-T., Lee, J.S., Park, M.-S., Choi, K.-H., Cho, K.-H., Kim, B.J., Park, J.-M., Kang, K., Lee, S.J., Kim, J.G., Cha, J.-K., Kim, D.-H., Park, T.H., Park, S.-S., Lee, K.B., Lee, Jun, Hong, K.-S., Cho, Y.-J., Park, H.-K., Lee, B.-C., Yu, K.-H., Sun Oh, M., Kim, D.-E., Ryu, W.-S., Choi, J.C., Kwon, J.-H., Kim, W.-J., Shin, D.-I., Sohn, S.I., Hong, J.-H., Lee, Juneyoung, Bae, H.-J., 2020. Comparative Effectiveness of Dual Antiplatelet Therapy With Aspirin and Clopidogrel Versus Aspirin Monotherapy in Mild-to-Moderate Acute Ischemic Stroke According to the Risk of Recurrent Stroke. Circulation: Cardiovascular Quality and Outcomes 13, e006474. https://doi.org/10.1161/CIRCOUTCOMES.119.006474
Lemežienė, N., Padarauskas, A., Butkutė, B., Cesevičienė, J., Taujenis, L., Norkevičienė, E., Mikaliūnienė, J., 2015. The concentration of isoflavones in red clover (Trifolium pratense L.) at flowering stage. Žemdirbystė (Agriculture) 102, 443–448.
Levick, J.R., 1991. Chapter 1 - Overview of the cardiovascular system, in: Levick, J.R. (Ed.), An Introduction to Cardiovascular Physiology. Butterworth-Heinemann, pp. 1–12. https://doi.org/10.1016/B978-0-7506-1028-5.50004-4
Levin, E.R., 2009. Plasma Membrane Estrogen Receptors. Trends Endocrinol Metab 20, 477–482. https://doi.org/10.1016/j.tem.2009.06.009

Li, B., Wang, J.H.-C., 2011. Fibroblasts and Myofibroblasts in Wound Healing: Force Generation and Measurement. J Tissue Viability 20, 108–120. https://doi.org/10.1016/j.jtv.2009.11.004

Li, C.I., Malone, K.E., Porter, P.L., Weiss, N.S., Tang, M.-T.C., Cushing-Haugen, K.L., Daling, J.R., 2003. Relationship Between Long Durations and Different Regimens of Hormone Therapy and Risk of Breast Cancer. JAMA 289, 3254–3263. https://doi.org/10.1001/jama.289.24.3254

Li, S.-H., Liu, X.-X., Bai, Y.-Y., Wang, X.-J., Sun, K., Chen, J.-Z., Hui, R.-T., 2010. Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. Am J Clin Nutr 91, 480–486. https://doi.org/10.3945/ajcn.2009.28203

Li, T.Y., Rana, J.S., Manson, J.E., Willett, W.C., Stampfer, M.J., Colditz, G.A., Rexrode, K.M., Hu, F.B., 2006. Obesity as Compared With Physical Activity in Predicting Risk of Coronary Heart Disease in Women. Circulation 113, 499–506. https://doi.org/10.1161/CIRCULATIONAHA.105.574087

Li, Z., Delaney, M.K., O'Brien, K.A., Du, X., 2010. Signaling during platelet adhesion and activation. Arterioscler Thromb Vasc Biol 30, 2341–2349. https://doi.org/10.1161/ATVBAHA.110.207522

Lievertz, R.W., 1987. Pharmacology and pharmacokinetics of estrogens. American Journal of Obstetrics and Gynecology 156, 1289–1293. https://doi.org/10.1016/0002-9378(87)90166-9

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. Clin Interv Aging 13, 757–772. https://doi.org/10.2147/CIA.S158513

Liu, D., Zhang, Z., Gladwell, W., Teng, C.T., 2003. Estrogen Stimulates Estrogen-Related Receptor α Gene Expression through Conserved Hormone Response Elements. Endocrinology 144, 4894–4904. https://doi.org/10.1210/en.2003-0432

Liu, Y., Ma, L., Yang, X., Bie, J., Li, D., Sun, C., Zhang, J., Meng, Y., Lin, J., 2019. Menopausal Hormone Replacement Therapy and the Risk of Ovarian Cancer: A Meta-Analysis. Frontiers in Endocrinology 10.

Lundberg Slingsby, M.H., Gliemann, L., Thrane, M., Rytter, N., Egelund, J., Chan, M.V., Armstrong, P.C., Warner, T.D., Hellsten, Y., 2018. Platelet responses to pharmacological and physiological interventions in middle-aged men with different habitual physical activity levels. Acta Physiol 223, e13028. https://doi.org/10.1111/apha.13028

Lundberg Slingsby, M.H., Nyberg, M., Egelund, J., Mandrup, C.M., Frikke-Schmidt, R., Kirkby, N.S., Hellsten, Y., 2017. Aerobic exercise training lowers platelet reactivity and improves platelet sensitivity to prostacyclin in pre- and postmenopausal women. J Thromb Haemost 15, 2419–2431. https://doi.org/10.1111/jth.13866

Macfarlane, R.G., 1964. An Enzyme Cascade in the Blood Clotting Mechanism, and its Function as a Biochemical Amplifier. Nature 202, 498–499. https://doi.org/10.1038/202498a0

Mackman, N., 2009. The Role of Tissue Factor and Factor VIIa in Hemostasis. Anesth Analg 108, 1447–1452. https://doi.org/10.1213/ane.0b013e31819bceb1

Maessen, M.F.H., Verbeek, A.L.M., Bakker, E.A., Thompson, P.D., Hopman, M.T.E., Eijsvogels, T.M.H., 2016. Lifelong Exercise Patterns and Cardiovascular Health. Mayo Clinic Proceedings 91, 745–754. https://doi.org/10.1016/j.mayocp.2016.02.028

Magnette, A., Chatelain, M., Chatelain, B., Ten Cate, H., Mullier, F., 2016. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. Thromb J 14, 49. https://doi.org/10.1186/s12959-016-0123-z

Mainini, G., Torella, M., Di Donna, M.C., Esposito, E., Ercolano, S., Correa, R., Cucinella, G., Stradella, L., Luisi, A., Basso, A., Cerreto, F.V., Cicatiello, R., Matteo, M., De Franciscis, P., 2013. Nonhormonal management of postmenopausal women: effects of a red clover based isoflavones supplementation on climacteric syndrome and cardiovascular risk serum profile. Clin Exp Obstet Gynecol 40, 337–341.

Mallidi, J., Lata, K., 2019. Role of Gender in Dual Antiplatelet Therapy After Acute Coronary Syndrome. Curr Atheroscler Rep 21, 34. https://doi.org/10.1007/s11883-019-0795-5

Mandrup, C.M., Egelund, J., Nyberg, M., Slingsby, M.H.L., Andersen, C.B., Løgstrup, S., Bangsbo, J., Suetta, C., Stallknecht, B., Hellsten, Y., 2017. Effects of high-intensity training on cardiovascular risk factors in premenopausal and postmenopausal women. American Journal of Obstetrics & Gynecology 216, 384.e1-384.e11. https://doi.org/10.1016/j.ajog.2016.12.017

Mann, T.N., Lamberts, R.P., Lambert, M.I., 2014. High responders and low responders: factors associated with individual variation in response to standardized training. Sports Med 44, 1113–1124. https://doi.org/10.1007/s40279-014-0197-3

Manolis, A.S., Manolis, T.A., Papadimitriou, P., Koulouris, S., Melita, H., 2013. Combined antiplatelet therapy: still a sweeping combination in cardiology. Cardiovasc Hematol Agents Med Chem 11, 136–167. https://doi.org/10.2174/1871525711311020009

Mari, D., Ogliari, G., Castaldi, D., Vitale, G., Bollini, E.M., Lio, D., 2008. Hemostasis and ageing. Immun Ageing 5, 12. https://doi.org/10.1186/1742-4933-5-12

Markopoulos, M.C., Kassi, E., Alexandraki, K.I., Mastorakos, G., Kaltsas, G., 2015. Hyperandrogenism after menopause. Eur J Endocrinol 172, R79-91. https://doi.org/10.1530/EJE-14-0468

Marrocco, J., McEwen, B.S., 2016. Sex in the brain: hormones and sex differences. Dialogues Clin Neurosci 18, 373–383.

Marsden, J., 2002. The menopause, hormone replacement therapy and breast cancer. The Journal of Steroid Biochemistry and Molecular Biology, Proceedings of the 15th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology “Recent Advances in Steroid Biochemistry and Molecular Biology” 83, 123–132. https://doi.org/10.1016/S0960-0760(02)00258-3

Matthews, K.A., Crawford, S.L., Chae, C.U., Everson-Rose, S.A., Sowers, M.F., Sternfeld, B., Sutton-Tyrrell, K., 2009. Are Changes in Cardiovascular Disease Risk Factors in Midlife Women due to Chronological Aging or to the Menopausal Transition? J Am Coll Cardiol 54, 2366–2373. https://doi.org/10.1016/j.jacc.2009.10.009

Mauri, L., Kereiakes, D.J., Yeh, R.W., Driscoll-Shempp, P., Cutlip, D.E., Steg, P.G., Normand, S.-L.T., Braunwald, E., Wiviott, S.D., Cohen, D.J., Holmes, D.R., Krucoff, M.W., Hermiller, J., Dauerman, H.L., Simon, D.I., Kandzari, D.E., Garratt, K.N., Lee, D.P., Pow, T.K., Ver Lee, P., Rinaldi, M.J., Massaro, J.M., 2014. Twelve or 30 Months of Dual Antiplatelet Therapy after Drug-Eluting Stents. New England Journal of Medicine 371, 2155–2166. https://doi.org/10.1056/NEJMoa1409312

McDonnell, D.P., Norris, J.D., 2002. Connections and Regulation of the Human Estrogen Receptor. Science 296, 1642–1644. https://doi.org/10.1126/science.1071884

Mercado, C.P., Kilic, F., 2010. Molecular Mechanisms of SERT in Platelets: Regulation of Plasma Serotonin Levels. Mol Interv 10, 231–241. https://doi.org/10.1124/mi.10.4.6

Merten, M., Chow, T., Hellums, J.D., Thiagarajan, P., 2000. A new role for P-selectin in shear-induced platelet aggregation. Circulation 102, 2045–2050. https://doi.org/10.1161/01.cir.102.17.2045

Merten, M., Pakala, R., Thiagarajan, P., Benedict, C.R., 1999. Platelet microparticles promote platelet interaction with subendothelial matrix in a glycoprotein IIb/IIIa-dependent mechanism. Circulation 99, 2577–2582. https://doi.org/10.1161/01.cir.99.19.2577

Miller, V.M., Black, D.M., Brinton, E.A., Budoff, M.J., Cedars, M.I., Hodis, H.N., Lobo, R.A., Manson, J.E., Merriam, G.R., Naftolin, F., Santoro, N., Taylor, H.S., Harman, S.M., 2009. Using basic science to design a clinical trial: baseline characteristics of women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). J Cardiovasc Transl Res 2, 228–239. https://doi.org/10.1007/s12265-009-9104-y

Miller, V.M., Duckles, S.P., 2008. Vascular Actions of Estrogens: Functional Implications. Pharmacological Reviews 60, 210–241. https://doi.org/10.1124/pr.107.08002

Miner, J., Hoffhines, A., 2007. The Discovery of Aspirin's Antithrombotic Effects. Tex Heart Inst J 34, 179–186.

Mitchell, J.A., Ali, F., Bailey, L., Moreno, L., Harrington, L.S., 2008. Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. Exp Physiol 93, 141–147. https://doi.org/10.1113/expphysiol.2007.038588

Mitchell, M.D., 1978. A sensitive radioimmunoassay for 6-keto-prostaglandin F1α: preliminary observations on circulating concentrations. Prostaglandins and Medicine 1, 13–21. https://doi.org/10.1016/0161-4630(78)90072-1

Montero, D., Lundby, C., 2017. Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. J Physiol 595, 3377–3387. https://doi.org/10.1113/JP273480

Moreau, K.L., Hildreth, K.L., 2014. Vascular Aging across the Menopause Transition in Healthy Women. Advances in Vascular Medicine 2014, 1–12. https://doi.org/10.1155/2014/204390

Moreau, K.L., Stauffer, B.L., Kohrt, W.M., Seals, D.R., 2013. Essential Role of Estrogen for Improvements in Vascular Endothelial Function With Endurance Exercise in Postmenopausal Women. J Clin Endocrinol Metab 98, 4507–4515. https://doi.org/10.1210/jc.2013-2183

Muscella, A., Stefàno, E., Marsigliante, S., 2020. The effects of exercise training on lipid metabolism and coronary heart disease. Am J Physiol Heart Circ Physiol 319, H76–H88. https://doi.org/10.1152/ajpheart.00708.2019

Naci, H., Ioannidis, J.P.A., 2013. Comparative effectiveness of exercise and drug interventions on mortality outcomes: metaepidemiological study. BMJ : British Medical Journal (Online) 347. https://doi.org/10.1136/bmj.f5577

Nadal, A., Díaz, M., Valverde, M.A., 2001. The Estrogen Trinity: Membrane, Cytosolic, and Nuclear Effects. Physiology 16, 251–255. https://doi.org/10.1152/physiologyonline.2001.16.6.251

Nauman, J., Tauschek, L.C., Kaminsky, L.A., Nes, B.M., Wisløff, U., 2017. Global Fitness Levels: Findings From a Web-Based Surveillance Report. Progress in Cardiovascular Diseases, Physical Activity, Exercise and Fitness in Health and Disease 60, 78–88. https://doi.org/10.1016/j.pcad.2017.01.009

Nelson, H.D., Humphrey, L.L., Nygren, P., Teutsch, S.M., Allan, J.D., 2002. Postmenopausal hormone replacement therapy: scientific review. JAMA 288, 872–881. https://doi.org/10.1001/jama.288.7.872

Nestel, P.J., Pomeroy, S., Kay, S., Komesaroff, P., Behrsing, J., Cameron, J.D., West, L., 1999. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. J Clin Endocrinol Metab 84, 895–898. https://doi.org/10.1210/jcem.84.3.5561

Nishiyama, S.K., Wray, D.W., Richardson, R.S., 2008. Aging affects vascular structure and function in a limb-specific manner. J Appl Physiol (1985) 105, 1661–1670. https://doi.org/10.1152/japplphysiol.90612.2008

Norgard, N.B., Abu-Fadel, M., 2009. Comparison of prasugrel and clopidogrel in patients with acute coronary syndrome undergoing percutaneous coronary intervention. Vasc Health Risk Manag 5, 873–882.

Novella, S., Heras, M., Hermenegildo, C., Dantas, A.P., 2012. Effects of Estrogen on Vascular Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology 32, 2035–2042. https://doi.org/10.1161/ATVBAHA.112.250308

Novensà, L., Novella, S., Medina, P., Segarra, G., Castillo, N., Heras, M., Hermenegildo, C., Dantas, A.P., 2011. Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ERα/ERβ balance in female mice. PLoS One 6, e25335. https://doi.org/10.1371/journal.pone.0025335

Nyberg, M., Blackwell, J.R., Damsgaard, R., Jones, A.M., Hellsten, Y., Mortensen, S.P., 2012. Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans. The Journal of Physiology 590, 5361–5370. https://doi.org/10.1113/jphysiol.2012.239053

Nyberg, M., Egelund, J., Mandrup, C.M., Andersen, C.B., Hansen, K.M.B.E., Hergel, I.-M.F., Valbak-Andersen, N., Frikke-Schmidt, R., Stallknecht, B., Bangsbo, J., Hellsten, Y., 2017. Leg vascular and skeletal muscle mitochondrial adaptations to aerobic high-intensity exercise training are enhanced in the early postmenopausal phase. J Physiol 595, 2969–2983. https://doi.org/10.1113/JP273871

Nyberg, M., Egelund, J., Mandrup, C.M., Nielsen, M.B., Mogensen, A.S., Stallknecht, B., Bangsbo, J., Hellsten, Y., 2016. Early Postmenopausal Phase Is Associated With Reduced Prostacyclin-Induced Vasodilation That Is Reversed by Exercise Training. Hypertension 68, 1011–1020. https://doi.org/10.1161/HYPERTENSIONAHA.116.07866

Nyberg, M., Seidelin, K., Andersen, T.R., Overby, N.N., Hellsten, Y., Bangsbo, J., 2014. Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise training. Am J Physiol Regul Integr Comp Physiol 306, R510-517. https://doi.org/10.1152/ajpregu.00539.2013

Nystoriak, M.A., Bhatnagar, A., 2018. Cardiovascular Effects and Benefits of Exercise. Front Cardiovasc Med 5, 135. https://doi.org/10.3389/fcvm.2018.00135

Ojeda, S.R., Prevot, V., Heger, S., Lomniczi, A., Dziedzic, B., Mungenast, A., 2003. The neurobiology of female puberty. Horm Res 60 Suppl 3, 15–20. https://doi.org/10.1159/000074495

Olvera Lopez, E., Ballard, B.D., Jan, A., 2022. Cardiovascular Disease, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Ozemek, C., Laddu, D.R., Lavie, C.J., Claeys, H., Kaminsky, L.A., Ross, R., Wisloff, U., Arena, R., Blair, S.N., 2018. An Update on the Role of Cardiorespiratory Fitness, Structured Exercise and Lifestyle Physical Activity in Preventing Cardiovascular Disease and Health Risk. Progress in Cardiovascular Diseases, Advances in Echocardiography 61, 484–490. https://doi.org/10.1016/j.pcad.2018.11.005

Palta, S., Saroa, R., Palta, A., 2014. Overview of the coagulation system. Indian J Anaesth 58, 515–523. https://doi.org/10.4103/0019-5049.144643

Park, Y.-M., Pereira, R.I., Erickson, C.B., Swibas, T.A., Kang, C., Van Pelt, R.E., 2017. Time since menopause and skeletal muscle estrogen receptors, PGC-1α, and AMPK. Menopause 24, 815–823. https://doi.org/10.1097/GME.0000000000000829

Parker, B.A., Kalasky, M.J., Proctor, D.N., 2010. Evidence for sex differences in cardiovascular aging and adaptive responses to physical activity. Eur J Appl Physiol 110, 235–246. https://doi.org/10.1007/s00421-010-1506-7

Parker, W.A.E., Orme, R.C., Hanson, J., Stokes, H.M., Bridge, C.M., Shaw, P.A., Sumaya, W., Thorneycroft, K., Petrucci, G., Porro, B., Judge, H.M., Ajjan, R.A., Rocca, B., Storey, R.F., 2019. Very-low-dose twice-daily aspirin maintains platelet inhibition and improves haemostasis during dual-antiplatelet therapy for acute coronary syndrome. Platelets 30, 148–157. https://doi.org/10.1080/09537104.2019.1572880

Pedersen, M.T., Nørregaard, L.B., Jensen, T.D., Frederiksen, A.S., Ottesen, L., Bangsbo, J., 2022. The effect of 5 years of team sport on elderly males' health and social capital-An interdisciplinary follow-up study. Health Sci Rep 5, e760. https://doi.org/10.1002/hsr2.760

Pedersen, M.T., Vorup, J., Bangsbo, J., 2018. Effect of a 26-month floorball training on male elderly's cardiovascular fitness, glucose control, body composition, and functional capacity. J Sport Health Sci 7, 149–158. https://doi.org/10.1016/j.jshs.2017.12.002

Periayah, M.H., Halim, A.S., Mat Saad, A.Z., 2017. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. Int J Hematol Oncol Stem Cell Res 11, 319–327.

Perry, M.-C., Dufour, C.R., Tam, I.S., B'chir, W., Giguère, V., 2014. Estrogen-related receptor-α coordinates transcriptional programs essential for exercise tolerance and muscle fitness. Mol Endocrinol 28, 2060–2071. https://doi.org/10.1210/me.2014-1281

Pfitscher, A., Reiter, E., Jungbauer, A., 2008. Receptor binding and transactivation activities of red clover isoflavones and their metabolites. J Steroid Biochem Mol Biol 112, 87–94. https://doi.org/10.1016/j.jsbmb.2008.08.007

Phillips, D.R., 1985. Receptors for Platelet Agonists, in: George, J.N., Nurden, A.T., Phillips, D.R. (Eds.), Platelet Membrane Glycoproteins. Springer US, Boston, MA, pp. 145–169. https://doi.org/10.1007/978-1-4684-4880-1_7

Pierce, G.L., Eskurza, I., Walker, A.E., Fay, T.N., Seals, D.R., 2011. Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults. Clin Sci (Lond) 120, 13–23. https://doi.org/10.1042/CS20100174

Pignatelli, P., De Biase, L., Lenti, L., Tocci, G., Brunelli, A., Cangemi, R., Riondino, S., Grego, S., Volpe, M., Violi, F., 2005. Tumor necrosis factor-α as trigger of platelet activation in patients with heart failure. Blood 106, 1992–1994. https://doi.org/10.1182/blood-2005-03-1247

Pilote, L., Raparelli, V., 2018. Participation of Women in Clinical Trials: Not Yet Time to Rest on Our Laurels∗. Journal of the American College of Cardiology 71, 1970–1972. https://doi.org/10.1016/j.jacc.2018.02.069

Pilšáková, L., Riečanský, I., Jagla, F., 2010. The physiological actions of isoflavone phytoestrogens. Physiol Res 59, 651–664. https://doi.org/10.33549/physiolres.931902

Polo-Kantola, P., Erkkola, R., Irjala, K., Pullinen, S., Virtanen, I., Polo, O., 1999. Effect of short-term transdermal estrogen replacement therapy on sleep: a randomized, double-blind crossover trial in postmenopausal women. Fertility and Sterility 71, 873–880. https://doi.org/10.1016/S0015-0282(99)00062-X

Pomeroy, J.E., Helfer, A., Bursac, N., 2020. Biomaterializing the Promise of Cardiac Tissue Engineering. Biotechnol Adv 42, 107353. https://doi.org/10.1016/j.biotechadv.2019.02.009

Prior, J.C., 2020. Women's reproductive system as balanced estradiol and progesterone actions—A revolutionary, paradigm-shifting concept in women's health. Drug Discovery Today: Disease Models, Special section on Skin disease and Women's health 32, 31–40. https://doi.org/10.1016/j.ddmod.2020.11.005

Prossnitz, E.R., Barton, M., 2011. The G-protein-coupled estrogen receptor GPER in health and disease. Nat Rev Endocrinol 7, 715–726. https://doi.org/10.1038/nrendo.2011.122

Puca, A.A., Carrizzo, A., Villa, F., Ferrario, A., Casaburo, M., Maciąg, A., Vecchione, C., 2013. Vascular ageing: the role of oxidative stress. Int J Biochem Cell Biol 45, 556–559. https://doi.org/10.1016/j.biocel.2012.12.024

Rademaker, M., 2001. Do women have more adverse drug reactions? Am J Clin Dermatol 2, 349–351. https://doi.org/10.2165/00128071-200102060-00001

Rahman, M.M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., Almikhlafi, M.A., Alghamdi, S.Q., Alruwaili, A.S., Hossain, M.S., Ahmed, M., Das, R., Emran, T.B., Uddin, M.S., 2022. Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules 27, 233. https://doi.org/10.3390/molecules27010233

Randolph, J.F., Zheng, H., Sowers, M.R., Crandall, C., Crawford, S., Gold, E.B., Vuga, M., 2011. Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. J Clin Endocrinol Metab 96, 746–754. https://doi.org/10.1210/jc.2010-1746

Reed, B.G., Carr, B.R., 2018. The Normal Menstrual Cycle and the Control of Ovulation. MDText.com, Inc., Massachusetts.

Reuter, I., 2012. Aging, Physical Activity, and Disease Prevention 2012. J Aging Res 2012, 373294. https://doi.org/10.1155/2012/373294

Ribeiro, I. c., de Lucena, E. g. p., Oliveira, A. s., Fernandes, P. t., 2022. Physical exercise in menopause: a review of physiological changes. Comparative Exercise Physiology 18, 275–279. https://doi.org/10.3920/CEP220009

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Adams, R.J., Berry, J.D., Brown, T.M., Carnethon, M.R., Dai, S., de Simone, G., Ford, E.S., Fox, C.S., Fullerton, H.J., Gillespie, C., Greenlund, K.J., Hailpern, S.M., Heit, J.A., Ho, P.M., Howard, V.J., Kissela, B.M., Kittner, S.J., Lackland, D.T., Lichtman, J.H., Lisabeth, L.D., Makuc, D.M., Marcus, G.M., Marelli, A., Matchar, D.B., McDermott, M.M., Meigs, J.B., Moy, C.S., Mozaffarian, D., Mussolino, M.E., Nichol, G., Paynter, N.P., Rosamond, W.D., Sorlie, P.D., Stafford, R.S., Turan, T.N., Turner, M.B., Wong, N.D., Wylie-Rosett, J., 2011. Heart Disease and Stroke Statistics—2011 Update. Circulation 123, e18–e209. https://doi.org/10.1161/CIR.0b013e3182009701

Rosano, G.M.C., Vitale, C., Fini, M., 2009. Cardiovascular aspects of menopausal hormone replacement therapy. Climacteric 12, 41–46. https://doi.org/10.1080/13697130903012306

Rosenberg, R.D., Rosenberg, J.S., 1984. Natural anticoagulant mechanisms. J Clin Invest 74, 1–6.

Ross, R., 1999. Atherosclerosis--an inflammatory disease. N Engl J Med 340, 115–126. https://doi.org/10.1056/NEJM199901143400207

Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A.A., Howard, B.V., Johnson, K.C., Kotchen, J.M., Ockene, J., Writing Group for the Women's Health Initiative Investigators, 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 288, 321–333. https://doi.org/10.1001/jama.288.3.321

Rossouw, J.E., Prentice, R.L., Manson, J.E., Wu, L., Barad, D., Barnabei, V.M., Ko, M., LaCroix, A.Z., Margolis, K.L., Stefanick, M.L., 2007. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 297, 1465–1477. https://doi.org/10.1001/jama.297.13.1465

Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noël C., Beaton, A.Z., Benjamin, E.J., Benziger, C.P., Bonny, Aimé, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J., Catapano, A.L., Chugh, S.S., Cooper, L.T., Coresh, J., Criqui, M., DeCleene, N., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Solà, J., Fowkes, G., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N., Koroshetz, W., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Temesgen, A.M., Mokdad, A., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Moraes de Oliveira, G., Otto, C., Owolabi, M., Pratt, M., Rajagopalan, S., Reitsma, M., Ribeiro, A.L.P., Rigotti, N., Rodgers, A., Sable, C., Shakil, S., Sliwa-Hahnle, K., Stark, B., Sundström, J., Timpel, P., Tleyjeh, I.M., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L., Murray, C., Fuster, V., Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noel C., Beaton, A., Benjamin, E.J., Benziger, C.P., Bonny, Aime, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J.R., Catapano, A.L., Chugh, S., Cooper, L.T., Coresh, J., Criqui, M.H., DeCleene, N.K., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Sola, J., Fowkes, F.G.R., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N.J., Koroshetz, W.J., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Misganaw, A.T., Mokdad, A.H., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Oliveira, G.M.M., Otto, C.M., Owolabi, M.O., Pratt, M., Rajagopalan, S., Reitsma, M.B., Ribeiro, A.L.P., Rigotti, N.A., Rodgers, A., Sable, C.A., Shakil, S.S., Sliwa, K., Stark, B.A., Sundström, J., Timpel, P., Tleyjeh, I.I., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L.J., Abbasi-Kangevari, M., Abdi, A., Abedi, A., Aboyans, V., Abrha, W.A., Abu-Gharbieh, E., Abushouk, A.I., Acharya, D., Adair, T., Adebayo, O.M., Ademi, Z., Advani, S.M., Afshari, K., Afshin, A., Agarwal, G., Agasthi, P., Ahmad, S., Ahmadi, S., Ahmed, M.B., Aji, B., Akalu, Y., Akande-Sholabi, W., Aklilu, A., Akunna, C.J., Alahdab, F., Al-Eyadhy, A., Alhabib, K.F., Alif, S.M., Alipour, V., Aljunid, S.M., Alla, F., Almasi-Hashiani, A., Almustanyir, S., Al-Raddadi, R.M., Amegah, A.K., Amini, S., Aminorroaya, A., Amu, H., Amugsi, D.A., Ancuceanu, R., Anderlini, D., Andrei, T., Andrei, C.L., Ansari-Moghaddam, A., Anteneh, Z.A., Antonazzo, I.C., Antony, B., Anwer, R., Appiah, L.T., Arabloo, J., Ärnlöv, J., Artanti, K.D., Ataro, Z., Ausloos, M., Avila-Burgos, L., Awan, A.T., Awoke, M.A., Ayele, H.T., Ayza, M.A., Azari, S., B, D.B., Baheiraei, N., Baig, A.A., Bakhtiari, A., Banach, M., Banik, P.C., Baptista, E.A., Barboza, M.A., Barua, L., Basu, S., Bedi, N., Béjot, Y., Bennett, D.A., Bensenor, I.M., Berman, A.E., Bezabih, Y.M., Bhagavathula, A.S., Bhaskar, S., Bhattacharyya, K., Bijani, A., Bikbov, B., Birhanu, M.M., Boloor, A., Brant, L.C., Brenner, H., Briko, N.I., Butt, Z.A., Caetano dos Santos, F.L., Cahill, L.E., Cahuana-Hurtado, L., Cámera, L.A., Campos-Nonato, I.R., Cantu-Brito, C., Car, J., Carrero, J.J., Carvalho, F., Castañeda-Orjuela, C.A., Catalá-López, F., Cerin, E., Charan, J., Chattu, V.K., Chen, S., Chin, K.L., Choi, J.-Y.J., Chu, D.-T., Chung, S.-C., Cirillo, M., Coffey, S., Conti, S., Costa, V.M., Cundiff, D.K., Dadras, O., Dagnew, B., Dai, X., Damasceno, A.A.M., Dandona, L., Dandona, R., Davletov, K., De la Cruz-Góngora, V., De la Hoz, F.P., De Neve, J.-W., Denova-Gutiérrez, E., Derbew Molla, M., Derseh, B.T.,

Desai, R., Deuschl, G., Dharmaratne, S.D., Dhimal, M., Dhungana, R.R., Dianatinasab, M., Diaz, D., Djalalinia, S., Dokova, K., Douiri, A., Duncan, B.B., Duraes, A.R., Eagan, A.W., Ebtehaj, S., Eftekhari, A., Eftekharzadeh, S., Ekholuenetale, M., El Nahas, N., Elgendy, I.Y., Elhadi, M., El-Jaafary, S.I., Esteghamati, S., Etisso, A.E., Eyawo, O., Fadhil, I., Faraon, E.J.A., Faris, P.S., Farwati, M., Farzadfar, F., Fernandes, E., Fernandez Prendes, C., Ferrara, P., Filip, I., Fischer, F., Flood, D., Fukumoto, T., Gad, M.M., Gaidhane, S., Ganji, M., Garg, J., Gebre, A.K., Gebregiorgis, B.G., Gebregzabiher, K.Z., Gebremeskel, G.G., Getacher, L., Obsa, A.G., Ghajar, A., Ghashghaee, A., Ghith, N., Giampaoli, S., Gilani, S.A., Gill, P.S., Gillum, R.F., Glushkova, E.V., Gnedovskaya, E.V., Golechha, M., Gonfa, K.B., Goudarzian, A.H., Goulart, A.C., Guadamuz, J.S., Guha, A., Guo, Y., Gupta, R., Hachinski, V., Hafezi-Nejad, N., Haile, T.G., Hamadeh, R.R., Hamidi, S., Hankey, G.J., Hargono, A., Hartono, R.K., Hashemian, M., Hashi, A., Hassan, S., Hassen, H.Y., Havmoeller, R.J., Hay, S.I., Hayat, K., Heidari, G., Herteliu, C., Holla, R., Hosseini, M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Househ, M., Huang, J., Humayun, A., Iavicoli, I., Ibeneme, C.U., Ibitoye, S.E., Ilesanmi, O.S., Ilic, I.M., Ilic, M.D., Iqbal, U., Irvani, S.S.N., Islam, S.M.S., Islam, R.M., Iso, H., Iwagami, M., Jain, V., Javaheri, T., Jayapal, S.K., Jayaram, S., Jayawardena, R., Jeemon, P., Jha, R.P., Jonas, J.B., Jonnagaddala, J., Joukar, F., Jozwiak, J.J., Jürisson, M., Kabir, A., Kahlon, T., Kalani, R., Kalhor, R., Kamath, A., Kamel, I., Kandel, H., Kandel, A., Karch, A., Kasa, A.S., Katoto, P.D.M.C., Kayode, G.A., Khader, Y.S., Khammarnia, M., Khan, M.S., Khan, M.N., Khan, M., Khan, E.A., Khatab, K., Kibria, G.M.A., Kim, Y.J., Kim, G.R., Kimokoti, R.W., Kisa, S., Kisa, A., Kivimäki, M., Kolte, D., Koolivand, A., Korshunov, V.A., Koulmane Laxminarayana, S.L., Koyanagi, A., Krishan, K., Krishnamoorthy, V., Kuate Defo, B., Kucuk Bicer, B., Kulkarni, V., Kumar, G.A., Kumar, N., Kurmi, O.P., Kusuma, D., Kwan, G.F., La Vecchia, C., Lacey, B., Lallukka, T., Lan, Q., Lasrado, S., Lassi, Z.S., Lauriola, P., Lawrence, W.R., Laxmaiah, A., LeGrand, K.E., Li, M.-C., Li, B., Li, S., Lim, S.S., Lim, L.-L., Lin, H., Lin, Z., Lin, R.-T., Liu, X., Lopez, A.D., Lorkowski, S., Lotufo, P.A., Lugo, A., M, N.K., Madotto, F., Mahmoudi, M., Majeed, A., Malekzadeh, R., Malik, A.A., Mamun, A.A., Manafi, N., Mansournia, M.A., Mantovani, L.G., Martini, S., Mathur, M.R., Mazzaglia, G., Mehata, S., Mehndiratta, M.M., Meier, T., Menezes, R.G., Meretoja, A., Mestrovic, T., Miazgowski, B., Miazgowski, T., Michalek, I.M., Miller, T.R., Mirrakhimov, E.M., Mirzaei, H., Moazen, B., Moghadaszadeh, M., Mohammad, Y., Mohammad, D.K., Mohammed, S., Mohammed, M.A., Mokhayeri, Y., Molokhia, M., Montasir, A.A., Moradi, G., Moradzadeh, R., Moraga, P., Morawska, L., Moreno Velásquez, I., Morze, J., Mubarik, S., Muruet, W., Musa, K.I., Nagarajan, A.J., Nalini, M., Nangia, V., Naqvi, A.A., Narasimha Swamy, S., Nascimento, B.R., Nayak, V.C., Nazari, J., Nazarzadeh, M., Negoi, R.I., Neupane Kandel, S., Nguyen, H.L.T., Nixon, M.R., Norrving, B., Noubiap, J.J., Nouthe, B.E., Nowak, C., Odukoya, O.O., Ogbo, F.A., Olagunju, A.T., Orru, H., Ortiz, A., Ostroff, S.M., Padubidri, J.R., Palladino, R., Pana, A., Panda-Jonas, S., Parekh, U., Park, E.-C., Parvizi, M., Pashazadeh Kan, F., Patel, U.K., Pathak, M., Paudel, R., Pepito, V.C.F., Perianayagam, A., Perico, N., Pham, H.Q., Pilgrim, T., Piradov, M.A.,

Pishgar, F., Podder, V., Polibin, R.V., Pourshams, A., Pribadi, D.R.A., Rabiee, N., Rabiee, M., Radfar, A., Rafiei, A., Rahim, F., Rahimi-Movaghar, V., Ur Rahman, M.H., Rahman, M.A., Rahmani, A.M., Rakovac, I., Ram, P., Ramalingam, S., Rana, J., Ranasinghe, P., Rao, S.J., Rathi, P., Rawal, L., Rawasia, W.F., Rawassizadeh, R., Remuzzi, G., Renzaho, A.M.N., Rezapour, A., Riahi, S.M., Roberts-Thomson, R.L., Roever, L., Rohloff, P., Romoli, M., Roshandel, G., Rwegerera, G.M., Saadatagah, S., Saber-Ayad, M.M., Sabour, S., Sacco, S., Sadeghi, M., Saeedi Moghaddam, S., Safari, S., Sahebkar, A., Salehi, S., Salimzadeh, H., Samaei, M., Samy, A.M., Santos, I.S., Santric-Milicevic, M.M., Sarrafzadegan, N., Sarveazad, A., Sathish, T., Sawhney, M., Saylan, M., Schmidt, M.I., Schutte, A.E., Senthilkumaran, S., Sepanlou, S.G., Sha, F., Shahabi, S., Shahid, I., Shaikh, M.A., Shamali, M., Shamsizadeh, M., Shawon, M.S.R., Sheikh, A., Shigematsu, M., Shin, M.-J., Shin, J.I., Shiri, R., Shiue, I., Shuval, K., Siabani, S., Siddiqi, T.J., Silva, D.A.S., Singh, J.A., Mtech, A.S., Skryabin, V.Y., Skryabina, A.A., Soheili, A., Spurlock, E.E., Stockfelt, L., Stortecky, S., Stranges, S., Suliankatchi Abdulkader, R., Tadbiri, H., Tadesse, E.G., Tadesse, D.B., Tajdini, M., Tariqujjaman, M., Teklehaimanot, B.F., Temsah, M.-H., Tesema, A.K., Thakur, B., Thankappan, K.R., Thapar, R., Thrift, A.G., Timalsina, B., Tonelli, M., Touvier, M., Tovani-Palone, M.R., Tripathi, A., Tripathy, J.P., Truelsen, T.C., Tsegay, G.M., Tsegaye, G.W., Tsilimparis, N., Tusa, B.S., Tyrovolas, S., Umapathi, K.K., Unim, B., Unnikrishnan, B., Usman, M.S., Vaduganathan, M., Valdez, P.R., Vasankari, T.J., Velazquez, D.Z., Venketasubramanian, N., Vu, G.T., Vujcic, I.S., Waheed, Y., Wang, Y., Wang, F., Wei, J., Weintraub, R.G., Weldemariam, A.H., Westerman, R., Winkler, A.S., Wiysonge, C.S., Wolfe, C.D.A., Wubishet, B.L., Xu, G., Yadollahpour, A., Yamagishi, K., Yan, L.L., Yandrapalli, S., Yano, Y., Yatsuya, H., Yeheyis, T.Y., Yeshaw, Y., Yilgwan, C.S., Yonemoto, N., Yu, C., Yusefzadeh, H., Zachariah, G., Zaman, S.B., Zaman, M.S., Zamanian, M., Zand, R., Zandifar, A., Zarghi, A., Zastrozhin, M.S., Zastrozhina, A., Zhang, Z.-J., Zhang, Y., Zhang, W., Zhong, C., Zou, Z., Zuniga, Y.M.H., Murray, C.J.L., Fuster, V., 2020. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology 76, 2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010

Sabra, A., Lawrence, M.J., Aubrey, R., Obaid, D.R., Chase, A.J., Smith, D., Thomas, P., Storton, S., Davies, G., Hawkins, K., Morris, K., Evans, P.A., 2017. Characterisation of clot microstructure properties in stable coronary artery disease | Open Heart [WWW Document]. URL https://openheart.bmj.com/content/4/2/e000562.abstract (accessed 9.18.22).

Sadler, J.E., 1998. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem 67, 395–424. https://doi.org/10.1146/annurev.biochem.67.1.395

Samuels, C., 2009. Sleep, recovery, and performance: the new frontier in high-performance athletics. Phys Med Rehabil Clin N Am 20, 149–159, ix. https://doi.org/10.1016/j.pmr.2008.10.009

Santoro, N., Epperson, C.N., Mathews, S.B., 2015. Menopausal Symptoms and Their Management. Endocrinol Metab Clin North Am 44, 497–515. https://doi.org/10.1016/j.ecl.2015.05.001

Santoro, N., Randolph, J.F., 2011. Reproductive Hormones and the Menopause Transition. Obstet Gynecol Clin North Am 38, 455–466. https://doi.org/10.1016/j.ogc.2011.05.004

Santos-Parker, J.R., Strahler, T.R., Vorwald, V.M., Pierce, G.L., Seals, D.R., 2017. Habitual aerobic exercise does not protect against micro- or macrovascular endothelial dysfunction in healthy estrogen-deficient postmenopausal women. J Appl Physiol (1985) 122, 11–19. https://doi.org/10.1152/japplphysiol.00732.2016

Sathyapalan, T., Aye, M., Rigby, A.S., Thatcher, N.J., Dargham, S.R., Kilpatrick, E.S., Atkin, S.L., 2018. Soy isoflavones improve cardiovascular disease risk markers in women during the early menopause. Nutr Metab Cardiovasc Dis 28, 691–697. https://doi.org/10.1016/j.numecd.2018.03.007

Savi, P., Nurden, P., Nurden, A.T., Levy-Toledano, S., Herbert, J.M., 1998. Clopidogrel: a review of its mechanism of action. Platelets 9, 251–255. https://doi.org/10.1080/09537109876799

Scharhag-Rosenberger, F., Meyer, T., Gässler, N., Faude, O., Kindermann, W., 2010. Exercise at given percentages of VO2max: heterogeneous metabolic responses between individuals. J Sci Med Sport 13, 74–79. https://doi.org/10.1016/j.jsams.2008.12.626

Schmaier, A.H., 2008. The elusive physiologic role of Factor XII. J Clin Invest 118, 3006–3009. https://doi.org/10.1172/JCI36617

Schmidt, J.F., Hansen, P.R., Andersen, T.R., Andersen, L.J., Hornstrup, T., Krustrup, P., Bangsbo, J., 2014. Cardiovascular adaptations to 4 and 12 months of football or strength training in 65- to 75-year-old untrained men. Scand J Med Sci Sports 24 Suppl 1, 86–97. https://doi.org/10.1111/sms.12217

Schreiber, S.N., Emter, R., Hock, M.B., Knutti, D., Cardenas, J., Podvinec, M., Oakeley, E.J., Kralli, A., 2004. The estrogen-related receptor α (ERRα) functions in PPARγ coactivator 1α (PGC-1α)-induced mitochondrial biogenesis. Proceedings of the National Academy of Sciences 101, 6472–6477. https://doi.org/10.1073/pnas.0308686101

Schuff-Werner, P., Steiner, M., Fenger, S., Gross, H.-J., Bierlich, A., Dreissiger, K., Mannuß, S., Siegert, G., Bachem, M., Kohlschein, P., 2013. Effective estimation of correct platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. Br J Haematol 162, 684–692. https://doi.org/10.1111/bjh.12443

Seals, D.R., Jablonski, K.L., Donato, A.J., 2011. Aging and vascular endothelial function in humans. Clinical Science 120, 357–375. https://doi.org/10.1042/CS20100476

Seidelin, K., Nyberg, M., Piil, P., Jørgensen, N.R., Hellsten, Y., Bangsbo, J., 2017. Adaptations with Intermittent Exercise Training in Post- and Premenopausal Women. Medicine & Science in Sports & Exercise 49, 96–105. https://doi.org/10.1249/MSS.0000000000001071

Shibata, S., Fujimoto, N., Hastings, J.L., Carrick-Ranson, G., Bhella, P.S., Hearon Jr., C.M., Levine, B.D., 2018. The effect of lifelong exercise frequency on arterial stiffness. The Journal of Physiology 596, 2783–2795. https://doi.org/10.1113/JP275301

Simoncini, T., Garibaldi, S., Fu, X.-D., Pisaneschi, S., Begliuomini, S., Baldacci, C., Lenzi, E., Goglia, L., Giretti, M.S., Genazzani, A.R., 2008. Effects of phytoestrogens derived from red clover on atherogenic adhesion molecules in human endothelial cells. Menopause 15, 542–550. https://doi.org/10.1097/gme.0b013e318156f9d6

Smith, E.S., McKay, A.K.A., Kuikman, M., Ackerman, K.E., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., Burke, L.M., 2022. Auditing the Representation of Female Versus Male Athletes in Sports Science and Sports Medicine Research: Evidence-Based Performance Supplements. Nutrients 14, 953. https://doi.org/10.3390/nu14050953

Smith, L.L., 2000. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? Med Sci Sports Exerc 32, 317–331. https://doi.org/10.1097/00005768-200002000-00011

Sowers, M.R., Eyvazzadeh, A.D., McConnell, D., Yosef, M., Jannausch, M.L., Zhang, D., Harlow, S., Randolph, J.F., 2008. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab 93, 3478–3483. https://doi.org/10.1210/jc.2008-0567

Spalding, A., Vaitkevicius, H., Dill, S., MacKenzie, S., Schmaier, A., Lockette, W., 1998. Mechanism of epinephrine-induced platelet aggregation. Hypertension 31, 603–607. https://doi.org/10.1161/01.hyp.31.2.603

Spina, R.J., Ogawa, T., Kohrt, W.M., Martin, W.H., Holloszy, J.O., Ehsani, A.A., 1993. Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol (1985) 75, 849–855. https://doi.org/10.1152/jappl.1993.75.2.849

Squadrito, F., Altavilla, D., Morabito, N., Crisafulli, A., D'Anna, R., Corrado, F., Ruggeri, P., Campo, G.M., Calapai, G., Caputi, A.P., Squadrito, G., 2002. The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. Atherosclerosis 163, 339–347. https://doi.org/10.1016/S0021-9150(02)00013-8

Staessen, J.A., Ginocchio, G., Thijs, L., Fagard, R., 1997. Conventional and ambulatory blood pressure and menopause in a prospective population study. J Hum Hypertens 11, 507–514. https://doi.org/10.1038/sj.jhh.1000476

Stokes, K.Y., Granger, D.N., 2012. Platelets: a critical link between inflammation and microvascular dysfunction. J Physiol 590, 1023–1034. https://doi.org/10.1113/jphysiol.2011.225417

Su, H.I., Freeman, E.W., 2009. Hormone changes associated with the menopausal transition. Minerva Ginecol 61, 483–489.

Thomas, P., Pang, Y., 2013. Protective actions of progesterone in the cardiovascular system: potential role of membrane progesterone receptors (mPRs) in mediating rapid effects. Steroids 78, 583–588. https://doi.org/10.1016/j.steroids.2013.01.003

Thompson, W.R., Sallis, R., Joy, E., Jaworski, C.A., Stuhr, R.M., Trilk, J.L., 2020. Exercise Is Medicine. American Journal of Lifestyle Medicine 14, 511–523. https://doi.org/10.1177/1559827620912192

Thorp, J.A., Walsh, S.W., Brath, P.C., 1988. Low-dose aspirin inhibits thromboxane, but not prostacyclin, production by human placental arteries. Am J Obstet Gynecol 159, 1381–1384. https://doi.org/10.1016/0002-9378(88)90560-1

Thorup, A.C., Lambert, M.N., Kahr, H.S., Bjerre, M., Jeppesen, P.B., 2015. Intake of Novel Red Clover Supplementation for 12 Weeks Improves Bone Status in Healthy Menopausal Women. Evid Based Complement Alternat Med 2015, 689138. https://doi.org/10.1155/2015/689138

Timmons, J.A., Knudsen, S., Rankinen, T., Koch, L.G., Sarzynski, M., Jensen, T., Keller, P., Scheele, C., Vollaard, N.B.J., Nielsen, S., Akerström, T., MacDougald, O.A., Jansson, E., Greenhaff, P.L., Tarnopolsky, M.A., van Loon, L.J.C., Pedersen, B.K., Sundberg, C.J., Wahlestedt, C., Britton, S.L., Bouchard, C., 2010. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. J Appl Physiol (1985) 108, 1487–1496. https://doi.org/10.1152/japplphysiol.01295.2009

Ting, Y., Jiang, Y., Ho, C.-T., Huang, Q., 2014. Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals. Journal of Functional Foods 7, 112–128. https://doi.org/10.1016/j.jff.2013.12.010

Tofas, T., Draganidis, D., Deli, C.K., Georgakouli, K., Fatouros, I.G., Jamurtas, A.Z., 2020. Exercise-Induced Regulation of Redox Status in Cardiovascular Diseases: The Role of Exercise Training and Detraining. Antioxidants 9, 13. https://doi.org/10.3390/antiox9010013

Trifan, G., Gorelick, P.B., Testai, F.D., 2021. Efficacy and Safety of Using Dual Versus Monotherapy Antiplatelet Agents in Secondary Stroke Prevention: Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. Circulation 143, 2441–2453. https://doi.org/10.1161/CIRCULATIONAHA.121.053782

Tripathi, M., Yen, P.M., Singh, B.K., 2020. Estrogen-Related Receptor Alpha: An Under-Appreciated Potential Target for the Treatment of Metabolic Diseases. Int J Mol Sci 21, 1645. https://doi.org/10.3390/ijms21051645

Troy, G.C., 1988. An Overview of Hemostasis. Veterinary Clinics of North America: Small Animal Practice 18, 5–20. https://doi.org/10.1016/S0195-5616(88)50003-7

van Beek, J.H.G.M., Kirkwood, T.B.L., Bassingthwaighte, J.B., 2016. Understanding the physiology of the ageing individual: computational modelling of changes in metabolism and endurance. Interface Focus 6, 20150079. https://doi.org/10.1098/rsfs.2015.0079

Verdoia, M., Suryapranata, H., Damen, S., Camaro, C., Benit, E., Barbieri, L., Rasoul, S., Liew, H.B., Polad, J., Ahmad, W. a. W., Zambahari, R., Lalmand, J., van der Schaaf, R.J., Koh, T.H., Timmermans, P., Dilling-Boer, D., Veenstra, L.F., Van't Hof, A.W.J., Lee, S.W.L., Roolvink, V., Ligtenberg, E., Postma, S., Kolkman, E.J.J., Brouwer, M.A., Kedhi, E., De Luca, G., 2021. Gender differences with short-term vs 12 months dual antiplatelet therapy in patients with acute coronary syndrome treated with the COMBO dual therapy stent: 2-years follow-up results of the

REDUCE trial. J Thromb Thrombolysis 52, 797–807. https://doi.org/10.1007/s11239-021-02439-x
Viggiani, M.T., Polimeno, L., Di Leo, A., Barone, M., 2019. Phytoestrogens: Dietary Intake, Bioavailability, and Protective Mechanisms against Colorectal Neoproliferative Lesions. Nutrients 11, 1709. https://doi.org/10.3390/nu11081709
Villena, J.A., Kralli, A., 2008. ERRα: a metabolic function for the oldest orphan. Trends Endocrinol Metab 19, 269–276. https://doi.org/10.1016/j.tem.2008.07.005
Vitale, D.C., Piazza, C., Melilli, B., Drago, F., Salomone, S., 2013. Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet 38, 15–25. https://doi.org/10.1007/s13318-012-0112-y
Vorup, J., Pedersen, M.T., Brahe, L.K., Melcher, P.S., Alstrøm, J.M., Bangsbo, J., 2017. Effect of small-sided team sport training and protein intake on muscle mass, physical function and markers of health in older untrained adults: A randomized trial. PLoS One 12, e0186202. https://doi.org/10.1371/journal.pone.0186202
Wagner, P.D., 2011. The critical role of VEGF in skeletal muscle angiogenesis and blood flow. Biochem Soc Trans 39, 1556–1559. https://doi.org/10.1042/BST20110646
Wallace, R.B., Sherman, B.M., Bean, J.A., Treloar, A.E., Schlabaugh, L., 1979. Probability of menopause with increasing duration of amenorrhea in middle-aged women. Am J Obstet Gynecol 135, 1021–1024. https://doi.org/10.1016/0002-9378(79)90729-4
Wang, G.-R., Zhu, Y., Halushka, P.V., Lincoln, T.M., Mendelsohn, M.E., 1998. Mechanism of platelet inhibition by nitric oxide: In vivo phosphorylation of thromboxane receptor by cyclic GMP-dependent protein kinase. Proc Natl Acad Sci U S A 95, 4888–4893.
Warlo, E.M.K., Arnesen, H., Seljeflot, I., 2019. A brief review on resistance to P2Y12 receptor antagonism in coronary artery disease. Thrombosis Journal 17, 11. https://doi.org/10.1186/s12959-019-0197-5
Warner, T.D., Armstrong, P.C., Chan, M.V., Knowles, R.B., 2016. The importance of endothelium-derived mediators to the efficacy of dual anti-platelet therapy. Expert Rev Hematol 9, 223–225. https://doi.org/10.1586/17474086.2016.1140035
Warner, T.D., Nylander, S., Whatling, C., 2011. Anti-platelet therapy: cyclo-oxygenase inhibition and the use of aspirin with particular regard to dual anti-platelet therapy. Br J Clin Pharmacol 72, 619–633. https://doi.org/10.1111/j.1365-2125.2011.03943.x
Weisel, J.W., Litvinov, R.I., 2017. Fibrin Formation, Structure and Properties. Subcell Biochem 82, 405–456. https://doi.org/10.1007/978-3-319-49674-0_13
Welt, C.K., McNicholl, D.J., Taylor, A.E., Hall, J.E., 1999. Female reproductive aging is marked by decreased secretion of dimeric inhibin. J Clin Endocrinol Metab 84, 105–111. https://doi.org/10.1210/jcem.84.1.5381
Wendelboe, A.M., Raskob, G.E., 2016. Global Burden of Thrombosis. Circulation Research 118, 1340–1347. https://doi.org/10.1161/CIRCRESAHA.115.306841

Westby, C.M., Weil, B.R., Greiner, J.J., Stauffer, B.L., Desouza, C.A., 2011. Endothelin-1 vasoconstriction and the age-related decline in endothelium-dependent vasodilatation in men. Clinical Science 120, 485–491. https://doi.org/10.1042/CS20100475

White, J.G., 1998. Use of the electron microscope for diagnosis of platelet disorders. Semin Thromb Hemost 24, 163–168. https://doi.org/10.1055/s-2007-995836

Whorton, A.R., Willis, C.E., Kent, R.S., Young, S.L., 1984. The role of calcium in the regulation of prostacyclin synthesis by porcine aortic endothelial cells. Lipids 19, 17–24. https://doi.org/10.1007/BF02534603

Wickham, K.A., Spriet, L.L., 2023. Food for Thought: Physiological Considerations for Nutritional Ergogenic Efficacy. Scandinavian Journal of Medicine & Science in Sports n/a. https://doi.org/10.1111/sms.14307

Wiik, A., Hellsten, Y., Berthelson, P., Lundholm, L., Fischer, H., Jansson, E., 2009. Activation of estrogen response elements is mediated both via estrogen and muscle contractions in rat skeletal muscle myotubes. Am J Physiol Cell Physiol 296, C215-220. https://doi.org/10.1152/ajpcell.00148.2008

Wilkerson, W.R., Sane, D.C., 2002. Aging and thrombosis. Semin Thromb Hemost 28, 555–568. https://doi.org/10.1055/s-2002-36700

Willhite, L.A., O'Connell, M.B., 2001. Urogenital Atrophy: Prevention and Treatment. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 21, 464–480. https://doi.org/10.1592/phco.21.5.464.34486

World Health Organization, 2022. Cardiovascular diseases [WWW Document]. URL https://www.who.int/health-topics/cardiovascular-diseases (accessed 10.12.22).

Woulfe, D., Yang, J., Brass, L., 2001. ADP and platelets: the end of the beginning. J Clin Invest 107, 1503–1505.

Xia, H., Dufour, C.R., Giguère, V., 2019. ERRα as a Bridge Between Transcription and Function: Role in Liver Metabolism and Disease. Frontiers in Endocrinology 10.

Xu, X., Wang, B., Ren, C., Hu, J., Greenberg, D.A., Chen, T., Xie, L., Jin, K., 2017. Age-related Impairment of Vascular Structure and Functions. Aging Dis 8, 590–610. https://doi.org/10.14336/AD.2017.0430

Xu, Y., Shen, Y., Chen, D., Zhao, P., Jiang, J., 2021. Efficacy and Safety of Dual Antiplatelet Therapy in Patients Undergoing Coronary Stent Implantation: A Systematic Review and Network Meta-Analysis. Journal of Interventional Cardiology 2021, e9934535. https://doi.org/10.1155/2021/9934535

Yaffe, K., Sawaya, G., Lieberburg, I., Grady, D., 1998. Estrogen Therapy in Postmenopausal WomenEffects on Cognitive Function and Dementia. JAMA 279, 688–695. https://doi.org/10.1001/jama.279.9.688

Yousefzadeh, M., Henpita, C., Vyas, R., Soto-Palma, C., Robbins, P., Niedernhofer, L., 2021. DNA damage-how and why we age? Elife 10, e62852. https://doi.org/10.7554/eLife.62852

Yuan, S.Y., Rigor, R.R., 2010. Structure and Function of Exchange Microvessels, Regulation of Endothelial Barrier Function. Morgan & Claypool Life Sciences.

Yun, S.-H., Sim, E.-H., Goh, R.-Y., Park, J.-I., Han, J.-Y., 2016. Platelet Activation: The Mechanisms and Potential Biomarkers. BioMed Research International 2016, e9060143. https://doi.org/10.1155/2016/9060143

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J., Lisheng, L., INTERHEART Study Investigators, 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 364, 937–952. https://doi.org/10.1016/S0140-6736(04)17018-9

Zhong, P.-Y., Shang, Y.-S., Bai, N., Ma, Y., Niu, Y., Wang, Z.-L., 2021. Efficacy and Safety of Very Short-Term Dual Antiplatelet Therapy After Drug-Eluting Stents Implantation for Acute Coronary Syndrome: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Front Cardiovasc Med 8, 660360. https://doi.org/10.3389/fcvm.2021.660360

CHAPTER 3 OBJECTIVES & HYPOTHESES

To explore the impact of exercise training and phytoestrogen supplementation on vascular health in postmenopausal women, three unique studies were designed. These projects are outlined in *Chapters 4, 6, and 7*. A review article is presented in *Chapter 5*, which provides the groundwork for *Chapter 6* and highlights key aspects that bridge the three research studies. The project-specific objectives and hypotheses are summarized below.

3.1 Study I – Objective & Hypothesis (Chapter 4)

Objective: To examine the effects of 8 weeks of high-intensity exercise training on platelet reactivity and prostacyclin sensitivity at rest and in response to *in-vitro* treatment with dual anti-platelet therapy (DAPT) in postmenopausal women.

Hypothesis: Exercise training would improve resting platelet prostacyclin sensitivity and potentiate the effects of DAPT on platelet aggregation in postmenopausal women.

3.2 Review I – Objective & Hypothesis (Chapter 5)

Objective: To discuss the current literature investigating the effects of aging and menopause on vascular health and to highlight the importance of timing of exercise training in aging women.

3.3 Study II – Objective & Hypothesis (Chapter 6)

Objective: To determine the impact of time after menopause on vascular adaptations to an 8-week high-intensity exercise training program.

Hypothesis: Recently postmenopausal women (≤5 years after menopause) would have more significant vascular adaptations to the exercise program compared to late postmenopausal women (≥10 years after menopause).

3.4 Study III – Objective & Hypothesis (Chapter 7)

Objective: To explore the effects of 2 weeks of fermented red clover extract supplementation on vascular health in recently postmenopausal women.

Hypothesis: Short-term supplementation with fermented red clover extract would significantly improve markers of vascular health in the blood and skeletal muscle of recently postmenopausal women.

CHAPTER 4

Study I – High-intensity exercise training improves basal platelet prostacyclin sensitivity and potentiates the response to dual anti-platelet therapy in postmenopausal women

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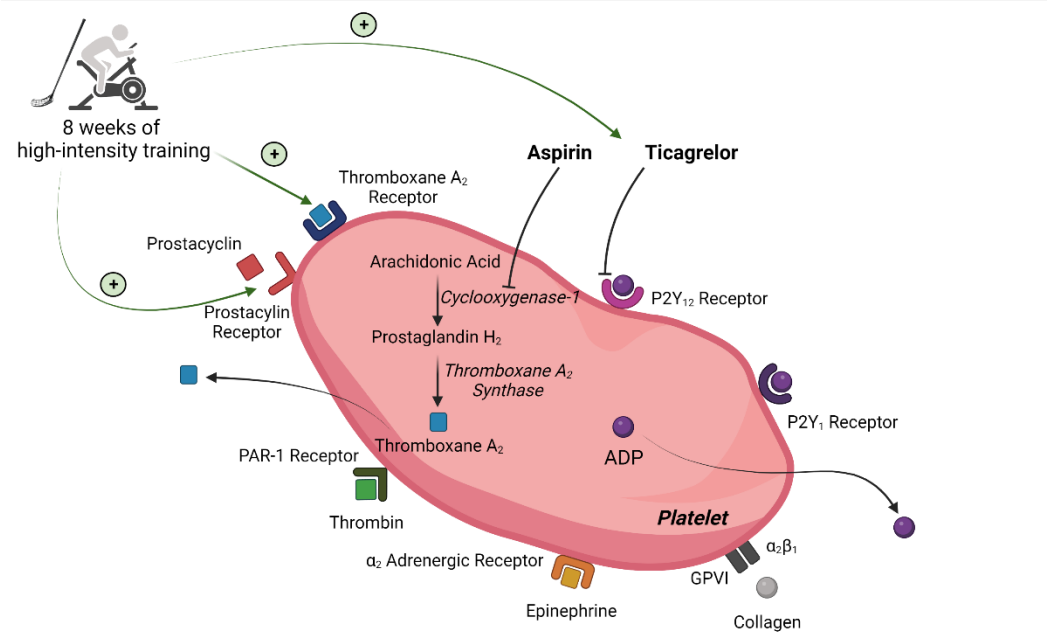

Figure 4-1: Study I graphical abstract.

8 weeks of high-intensity exercise training significantly improves basal platelet prostacyclin sensitivity and in response to dual anti-platelet therapy consisting of aspirin and ticagrelor.

4.1 Abstract

The risk of thrombotic events dramatically increases with age and may be accelerated in women by the cessation of endogenous estrogen production at menopause. Patients at risk of thrombosis are prescribed dual anti-platelet therapy (DAPT; aspirin and a $P2Y_{12}$ antagonist) and are encouraged to participate in regular physical activity, as these modalities improve nitric oxide and prostacyclin-mediated inhibition of platelet aggregation.

Methods: We assessed prostacyclin sensitivity as well as basal platelet reactivity with and without *in-vitro* DAPT before and after an 8-week high-intensity exercise training program in 13 healthy, sedentary postmenopausal women. The training intervention consisted of three 1-hour sessions per week. Isolated platelets were analyzed for thromboxane A_2 receptor, thromboxane A_2 synthase, cyclooxygenase-1, and prostacyclin receptor protein expression. Additionally, plasma 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 levels were determined.

Results: Exercise training made platelets more sensitive to the inhibitory effects of prostacyclin on thromboxane-, collagen-, and adenosine 5'-diphosphate (ADP)-induced aggregation, as well as thrombin-receptor activator peptide 6- and ADP-induced aggregation with DAPT. However, there was no change in protein expression from isolated platelets or plasma thromboxane B_2 and prostacyclin levels following training.

Conclusion: Together, these findings emphasize the importance of promoting physical activity as a tool for reducing thrombotic risk in postmenopausal women and suggest that training status should be considered when prescribing DAPT in this cohort.

4.2 Introduction

Thrombotic events are a leading cause of death, affecting one in four people worldwide [1], and the risk of thrombosis dramatically increases after the age of 60 [2]. In healthy individuals, circulating platelets are activated in response to vascular injury to promote platelet aggregation and subsequent thrombus formation [3]. However, aging is associated with an increase in circulating factors that promote platelet activation without a proportional increase in factors that prevent platelet activation, contributing to an increased thrombotic risk [4–6]. In a large cohort study of men and women, each successive decade of life was linked to an ~8% increase in platelet aggregability [7]. Notably, compared to men, women may have an exaggerated increase in platelet hyperreactivity and thrombotic risk with age due to menopause, and the concomitant loss of endogenous estrogen [8–10]. Estrogen inhibits platelet reactivity by stimulating the production of key endothelial-derived platelet inhibitors including nitric oxide (NO) [11] and prostacyclin [12]. Therefore, in menopause, when estrogen levels are very low, the balance is shifted in favour of platelet activation over inactivation, and thus blood clot formation, which contributes to the elevated risk of thrombosis in this cohort [8,10].

Patients who have experienced a thrombotic event, or who are deemed at risk of thrombosis, are typically prescribed dual anti-platelet therapy (DAPT) for 12 months [13]

to reduce platelet hyperreactivity and the formation of harmful blood clots. DAPT also dramatically increases the anti-platelet effects of NO and prostacyclin [14–16]. DAPT consists of aspirin, which inhibits platelet production of thromboxane A_2 and a $P2Y_{12}$ receptor antagonist (e.g., ticagrelor, prasugrel, or clopidogrel), which inhibits the activation of platelet adenosine 5'-diphosphate (ADP) $P2Y_{12}$ receptors. In addition to DAPT, the patients are encouraged to participate in regular physical activity, due to its established array of beneficial effects on vascular health [13,17,18]. Importantly, in postmenopausal women, regular exercise mimics many of the same cardioprotective effects as endogenous estrogen, and the reductions in platelet hyperreactivity are at least partially attributed to exercise-induced improvements in NO and prostacyclin bioavailability [19–21] and platelet prostacyclin sensitivity [9]. Taken together, regular exercise and DAPT both elicit anti-platelet effects via NO and prostacyclin-mediated pathways, highlighting the potential for a synergistic effect.

Previous research from our laboratory has shown that well-trained middle-aged men are more sensitive to DAPT (aspirin and ticagrelor) than age-matched untrained individuals [22]. This demonstrates that in men, training status should be considered when prescribing DAPT. However, given that current health guidelines recommend concomitant DAPT and regular physical activity interventions [13], which both provide potent anti-platelet effects, it is critical to evaluate the direct impact of an exercise training program on the efficacy of DAPT in previously sedentary individuals. Moreover, several reviews have recently drawn attention to the severe underrepresentation of women in DAPT research initiatives [23,24], as well as preliminary evidence for lower

DAPT efficacy in women compared to men [25,26]. Therefore, the purpose of this study is to assess the impact of an 8-week high-intensity exercise training program on: (**1**) prostacyclin sensitivity with and without *in-vitro* DAPT, (**2**) basal platelet reactivity with and without *in-vitro* DAPT, (**3**) isolated platelet protein expression, and (**4**) circulating levels of platelet activators and inhibitors in postmenopausal women. We hypothesized that 8 weeks of high-intensity exercise training would improve platelet prostacyclin sensitivity with and without *in-vitro* DAPT, which would be mediated by increases in platelet prostacyclin receptor expression and circulating prostacyclin.

4.3 Methods

The experimental protocol was approved by the ethics committee of Copenhagen (H-20037633). All participants provided written informed consent and experiments were conducted in accordance with the Declaration of Helsinki.

4.3.1 Recruitment of Subjects

Sixteen participants were recruited for an 8-week exercise training intervention via an online participant database and newspaper advertisements. The inclusion criteria were sedentary (no regular physical activity in >2 years) but otherwise healthy, postmenopausal women (>12 months since last menstrual bleeding, confirmed by blood hormone levels; Figure 4-2), 50 to 70 years old, body mass index (BMI) <30 $kg \cdot m^{-2}$, and normotensive (blood pressure <130/90 mmHg). Participants were excluded if they smoked in the last 10 years or had excessive alcohol intake (>14 units per week as well as blood alanine transferase and aspartate aminotransferase levels). Other exclusion criteria

were the use of regular medication, hormone replacement therapy, or phytoestrogen supplements (e.g., soybean or red clover products).

4.3.2 Study Design

The study design consisted of a health screening day, two body composition days (pre- and post-training), two experimental days (pre- and post-training), and 8 weeks of high-intensity exercise training. For all laboratory visits, participants were instructed to avoid caffeine for 24 hours, strenuous exercise for 48 hours, and non-steroidal anti-inflammatory drugs for at least 7 days prior to participation.

4.3.3 Health Screening Day

All participants underwent an examination to ascertain their health status, eligibility to participate in exercise training, and that all inclusion criteria were met. The health examination included: a 10-point resting electrocardiogram (ECG), blood samples for hematological markers, sex hormones, and cholesterol (analyzed within 2 hours at Rigshospitalet in Copenhagen, Denmark) (Figures 4-2 and 4-3). Participants also completed the short version of the International Physical Activity Questionnaire (IPAQ) to quantify their self-reported physical activity habits.

4.3.4 Exercise Training

The participants underwent 8 weeks of high-intensity exercise training, which involved 1 hour of training performed 3 times per week. The training program was accepted as completed when participants performed a minimum of 20 sessions, and training was terminated at a maximum of 26 sessions over the 8-week period. The training included a

combination of small-sided floorball matches and interval-based cycle training (spinning). This mixed modality, high-intensity training program was selected to maintain participant motivation and to avoid overuse injuries. Participants were required to participate in at least one session of floorball and one session of spinning per week with the option to select the third session based on preference. Each training session was monitored by members of the research team and encouragement was provided to facilitate high-intensity exercise. Additionally, heart rate (HR) was monitored continuously (POLAR TEAM Pro, Polar Electro Oy, Finland) throughout the exercise sessions to quantitatively ensure a high exercise intensity was maintained (average HR of the entire session >75% HRmax). HR data was quantified using time spent in HR zones, where Zone 1 was ≤60% HRmax, Zone 2 was 61 to 70% HRmax, Zone 3 was 71 to 80% HRmax, Zone 4 was 81 to 90% HRmax, Zone 5 was ≥91% HRmax, as defined by POLAR TEAM Pro (Polar Electro Oy, Finland).

4.3.5 Body Composition Days

Before and after the training intervention, participants arrived at the laboratory in a fasted state and rested in a supine position for 15 minutes before a dual energy x-ray absorptiometry (DXA) scan was performed to assess body composition.

4.3.6 Experimental Days

Experimental days were performed pre- and post-training. The participants arrived at the laboratory in a semi-fasted state (≥2 hours since last meal) and were instructed to repeat their diet for the post-training experimental day. A diet record was utilized to ensure successful repetition. Then, resting blood samples were drawn into 3.2% sodium citrate

tubes (454332, Greiner Bio-One, Austria) for basal and prostacyclin sensitivity platelet reactivity assays (~34 mL) and CTAD tubes (~18 mL; 454064, Greiner Bio-One, Austria) for platelet isolation. Participants then performed a maximal oxygen uptake ($\dot{V}O_{2max}$) test on a cycle ergometer for determination of fitness status. The test consisted of a 5 min warm-up at 50 Watts (W) followed by 25 $W \cdot min^{-1}$ increases until volitional exhaustion. Participants then rested for 10 min before completing a $\dot{V}O_{2max}$ verification at 110% W_{max} until volitional exhaustion.

4.3.7 Basal Platelet Reactivity Assay

The primary outcomes of this assay were to: **(1)** determine the effects of exercise training on platelet aggregation and **(2)** evaluate the anti-aggregatory effects of DAPT pre- and post-training. To perform this assay, 18 mL of blood were drawn into 3.2% sodium citrate tubes. The blood was immediately analyzed for mean platelet count, red blood cells (RBC), and white blood cells (WBC) using a hematology analyzer (Sysmex XN-2100, Sysmex, Japan). Blood samples were then centrifuged at 180 g for 10 min at 20°C to obtain platelet rich plasma (PRP). The PRP was pipetted into two 2 mL aliquots for treatment with vehicle or DAPT. The remaining blood was centrifuged further at 15000 g for 2 min at 20°C to obtain platelet poor plasma (PPP). One 2 mL aliquot of PRP was treated with vehicle consisting of 0.5% DMSO and 0.03% ethanol in 1x phosphate buffered saline (PBS, Gibco PBS (10X), pH 7.2, Fisher Scientific, USA). The second 2 mL aliquot of PRP was treated with DAPT consisting of the reversible purinergic $P2Y_{12}$ receptor antagonist Ticagrelor (1 µM; 15425, Cayman Chemical, USA) and aspirin (acetyl-salicylic acid, 100 µM; 5376, Sigma-Aldrich, USA) [22,27] at concentrations that reflect *in-vivo* platelet reactivity in the

presence of DAPT [28,29]. The PRP aliquots were incubated for 30 min at 37°C in a water bath. The PRP and PPP were then pipetted into a 96-well round bottom plate (650101, Greiner Bio-One, Austria) and, as per our previous studies [9,22], 40 µL was subsequently added via multi-channel pipette to a 96-well half-area plate (675161, Greiner Bio-One, Austria) pre-coated with known concentrations of platelet agonists: collagen [0.0156 – 16 µg·mL-1; 1130630, Takeda, Japan], thrombin receptor activator peptide 6 [0.11 – 40 µM; TRAP-6, 4017752, Bachem, Switzerland], epinephrine [0.001 – 10 µM; E4375, Sigma-Aldrich, USA], ADP [0.08 – 80 µM; A2754, Sigma-Aldrich, USA], and thromboxane A_2 mimetic U46619 [0.02 – 40 µM; 16450, Cayman Chemical Company, USA]. The 96-well half-area plate was covered with Parafilm and put on an orbital plate shaker (Thermomixer C, Eppendorf, Germany) for 5 min at 37°C and 1200 rpm to allow aggregation to occur. The 96-well half-area plate was immediately analyzed using a plate reader (Emax, Molecular Devices, USA), where absorbance was measured at 595 nm and platelet aggregation (%) was calculated:

$$\boldsymbol{Platelet\ aggregation}\ (\%) = 1 - \left(\frac{sample - PPP}{PRP - PPP}\right) x\ 100\%$$

4.3.8 Prostacyclin Sensitivity Assay

The primary outcomes of this assay were to: **(1)** determine the effects of exercise training on the anti-aggregatory action of prostacyclin and **(2)** evaluate the combined anti-aggregatory effects of prostacyclin and DAPT pre- and post-training. To perform this assay, 16 mL of blood drawn into 3.2% sodium citrate tubes was used. The centrifugation steps and treatment with vehicle or DAPT was the same was the basal platelet reactivity

assay. The PRP was then pipetted into aliquots on a 96-well round bottom plate (650101, Greiner Bio-One, Austria) and, to mimic a physiological milieu, was treated with low concentrations of prostacyclin [1 – 300 nM; epoprostenol 2989, R&D Systems, USA] [22] for 1 min at room temperature before 40 µL of this treated PRP was added via multi-channel pipette to a 96-well half-area plate (675161, Greiner Bio-One, Austria) pre-coated with known concentrations of platelet agonists: collagen [10 µg·mL-1], TRAP-6 [10 µM], ADP [20 µM], and U46619 [10 µM]. The 96-well half-area plate was covered with Parafilm and underwent the same shaking and absorbance measurement as previously described. All assays were completed within 2 hours of drawing the blood.

4.3.9 Platelet Isolation

Blood drawn into CTAD tubes (18 mL) was centrifuged for 10 min at 180 g and 20°C to obtain PRP, which was transferred to a 15 mL tube then treated with 1 µL prostaglandin E1 (P5515, Sigma-Aldrich, USA) per 100 µL PRP to reversibly inhibit platelet activation. The PRP was centrifuged for 5 min at 5000 g and 20°C to form a platelet pellet. The supernatant was removed and platelet wash buffer (140 mM NaCl, 5 mM KCl, 12 mM sodium citrate, 10 mM glucose, 12.5 mM sucrose, pH 6.0) was added in equal volumes to the initial volume of PRP. The pellet and wash buffer were centrifuged for 5 min at 5000 g and 20°C. The supernatant was removed, and the platelet pellet was resuspended in 400 µL of platelet resuspension buffer (10 mM HEPES, 140 mM NaCl, 3 mM KCl, 0.5 mM $MgCl_2$, 5 mM $NaHCO_3$, 10 mM glucose, pH 7.4). The platelet count was obtained (Sysmex XN-2100, Sysmex, Japan), then an equal volume of 0.5% Triton X-100 (T8787, Sigma-Aldrich, USA) in 1x phosphate-buffered saline (PBS) was added to the resuspended

platelets to ensure lysis prior to storage at -80°C. Platelet isolation was initiated within 3 hours of drawing the blood.

4.3.10 Western Blotting of Isolated Platelets

Platelet cell lysates were prepared for western blot analysis by adding concentrated sample buffer (0.5 M Tris-base, DTT, SDS, glycerol, and bromophenol blue) and heated for 3 min at 96°C prior to loading. Approximately $1.9x10^6$ platelets were loaded per well using pre-cast Criterion TGX stain-free gels (4-15%) (Bio-Rad, USA). Stain-free (TGX) images of the gels were obtained as a loading control. Then, proteins were semi-dry transferred to a polyvinylidene difluoride membrane (Immobilon Transfer Membrane, Millipore, USA). The membranes were incubated overnight at 4°C with primary antibodies diluted in either 5% milk (thromboxane A_2 receptor; ab233288, 1:1000 and cyclooxygenase-1; ab133319, 1:2000) or 3% BSA (thromboxane A_2 synthase; ab39362, 1:1000 and prostacyclin receptor; ab196653, 1:1000). Next, the membranes were washed for 5 min with TBST before adding anti-rabbit secondary horseradish peroxidase conjugated antibody (111-035-144, Jackson Immunoresearch, USA) for 1 hour. Bands were visualized with Luminata Forte (Merck Millipore, Germany). The images were digitized on a ChemiDoc MP system (Bio-Rad, USA). All proteins were expressed in arbitrary units normalized to the average of all samples loaded on the gel.

4.3.11 Plasma Thromboxane B_2 and 6-Keto Prostaglandin $F_{1\alpha}$ Assays

Three mL of blood was collected in an EDTA-coated tube and was immediately centrifuged at 4000 rpm for 5 min at 5°C. Plasma was stored at -80°C until future analysis. Plasma concentrations of prostacyclin and thromboxane A_2 were determined via their

breakdown products, 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) and thromboxane B_2, respectively. The thromboxane B_2 (KGE011, R&D Systems, USA) and 6-keto $PGF_{1\alpha}$ (515211, Cayman Chemical, USA) assays were performed according to the manufacturer's instructions.

4.3.12 Statistical Analyses

The sample size of 15 participants was determined by performing a power calculation with an α level of 0.05 and a power level of 0.8 for a 50% increase in platelet sensitivity to the inhibitory effects of prostacyclin, based on previous reports [9,22,30]. We recruited 16 participants, and the final data set includes 13 participants due to 3 dropouts (COVID-19, injury, and inability to commit to the training program). Statistical analyses were performed using R-studio (Version 4.1.2, R Foundation for Statistical Computing, Austria). Figures were created using GraphPad Prism (GraphPad Software, Version 9.3.1, USA). Basal platelet reactivity and prostacyclin sensitivity were performed using linear mixed models with a Tukey post-hoc test. The half maximal effective concentration (EC_{50}) was determined as the concentration of agonist required to elicit half of the maximal platelet aggregation. The half maximal inhibitory concentration (IC_{50}) was determined as the concentration of prostacyclin required to elicit half of the maximal inhibition of platelet aggregation. The effects of exercise training on health parameters, EC_{50}, IC_{50}, platelet western blots, and ELISA parameters were analyzed using a paired two-tailed t-test. Normality of the data was confirmed using Q-Q plots. Statistical significance was accepted at $p \leq 0.05$. Data are reported as mean ± standard deviation (SD). The final number of replicates is indicated in each figure legend.

4.4 Results

4.4.1 Participant Characteristics

Thirteen healthy, but sedentary, postmenopausal women completed the 8-week high-intensity training intervention. A summary of the participant characteristics can be found in Figure 4-2.

Participant Characteristics	
Age (years)	61 ± 5
Years After Menopause	10 ± 6
Height (cm)	166 ± 5
Estradiol ($nmol \cdot L^{-1}$)	0.09 ± 0.00
Progesterone ($IU \cdot L^{-1}$)	0.6 ± 0.0
Testosterone ($nmol \cdot L^{-1}$)	0.4 ± 0.1
LH ($IU \cdot L^{-1}$)	36.0 ± 15.2
FSH ($IU \cdot L^{-1}$)	67.2 ± 25.6

Figure 4-2: Baseline characteristics of the study participants (n=13).

There was no statistically significant increase in absolute (mL $O_2 \cdot min^{-1}$; $p = 0.15$) or relative (mL $O_2 \cdot kg^{-1} \cdot min^{-1}$; $p = 0.14$) $\dot{V}O_{2max}$. However, after the training period, the participants showed a significant increase in lean body mass (LBM; $p = 0.01$), in addition to a significant reduction in relative fat mass ($p = 0.02$) and an increase in relative lean mass ($p = 0.02$). An overview of the body composition, hematological, as well as health and fitness parameters pre- and post-training is provided in Figure 4-3.

	Pre-Training	Post-Training	Statistical Significance
Body Composition Parameters			
Body Mass (kg)	72.2 ± 9.0	72.2 ± 8.6	$p = 0.92$
Lean Body Mass (kg)	40.9 ± 3.9	41.5 ± 3.5	$p = 0.01$ *
Fat Mass (kg)	28.8 ± 5.8	28.3 ± 5.7	$p = 0.16$
Lean Mass (%)	60.2 ± 4.4	61.1 ± 4.1	$p = 0.02$ *
Fat Mass (%)	39.8 ± 4.4	38.9 ± 4.1	$p = 0.02$ *
Hematological Parameters			
Platelet Count ($x10^3$)	180 ± 31	182 ± 28	$p = 0.76$
Red Blood Cells ($x10^6$)	5.2 ± 0.9	5.1 ± 1.3	$p = 0.65$
White Blood Cells ($x10^6$)	4.2 ± 0.3	4.4 ± 0.3	$p = 0.06$
Health and Fitness Parameters			
VO_{2max} ($mL \cdot min^{-1}$)	1895 ± 295	1956 ± 298	$p = 0.15$
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	26.6 ± 5.0	27.4 ± 4.8	p = 0.14
Total Cholesterol ($mmol \cdot L^{-1}$)	6.1 ± 0.7	5.7 ± 0.7	$p = 0.07$
Body Mass Index ($kg \cdot m^{-2}$)	26.0 ± 2.0	26.1 ± 2.0	$p = 0.74$

Figure 4-3: Body composition, hematological, and fitness adaptations to 8 weeks of high-intensity exercise training in postmenopausal women (n=13).

4.4.2 Exercise Training

The participants completed a total of 23 ± 2 training sessions over the 8-week period, of which 9 ± 3 sessions were spinning, and 13 ± 3 sessions were floorball training. The average training session was 53 min 56 sec ± 1 min 5 sec. Time spent in the different HR zones over the exercise sessions is depicted in Figure 4-4.

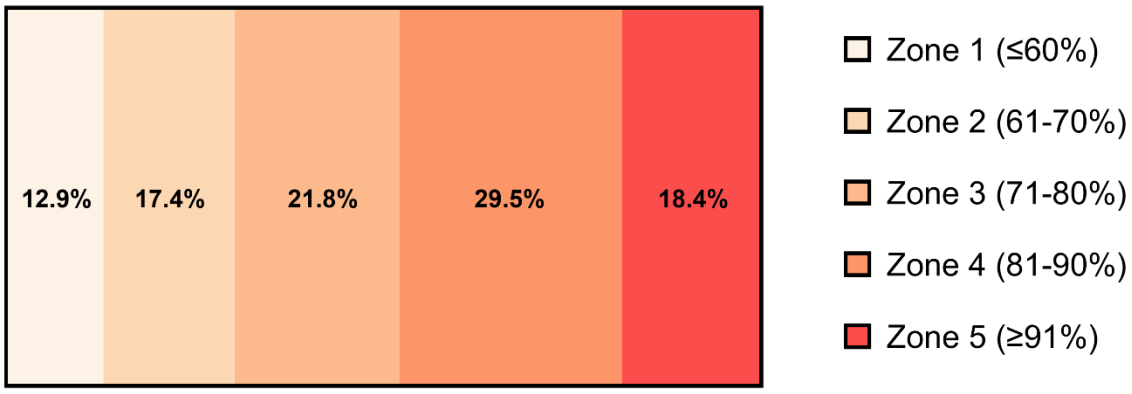

Figure 4-4: Average time spent in each training zone (%) over the 8-week high-intensity exercise training period (n=13).

The training zones were pre-defined by POLAR TEAM Pro. Zone 1 was ≤60% HR_{max}, Zone 2 was 61 to 70% HR_{max}, Zone 3 was 71 to 80% HR_{max}, Zone 4 was 81 to 90% HR_{max}, and Zone 5 was ≥91% HR_{max} (Polar Electro Oy, Finland).

4.4.3 Exercise Training and Basal Platelet Reactivity

Basal platelet reactivity was evaluated as the aggregation (%) to a known concentration of platelet agonist as well as the concentration of agonist required to elicit half of the maximal platelet aggregation (EC_{50}). There was a main effect of concentration, whereby increasing concentrations of collagen, TRAP6, ADP, U46619, and epinephrine resulted in higher levels of platelet aggregation ($p < 0.0001$ for all agonists). The exercise intervention increased platelet aggregation by ~28% to U46619 [1.98 µM], a thromboxane receptor analogue (65.8 ± 24.9 vs. 84.5 ± 7.6% aggregation; $p = 0.0002$) (Figures 4-5C, 4-5D) (lower

EC_{50}; p = 0.04; Figure 4-6E). However, the training intervention did not affect platelet aggregation or the EC_{50} to collagen, TRAP6, ADP, or epinephrine (Figures 4-5 and 4-6).

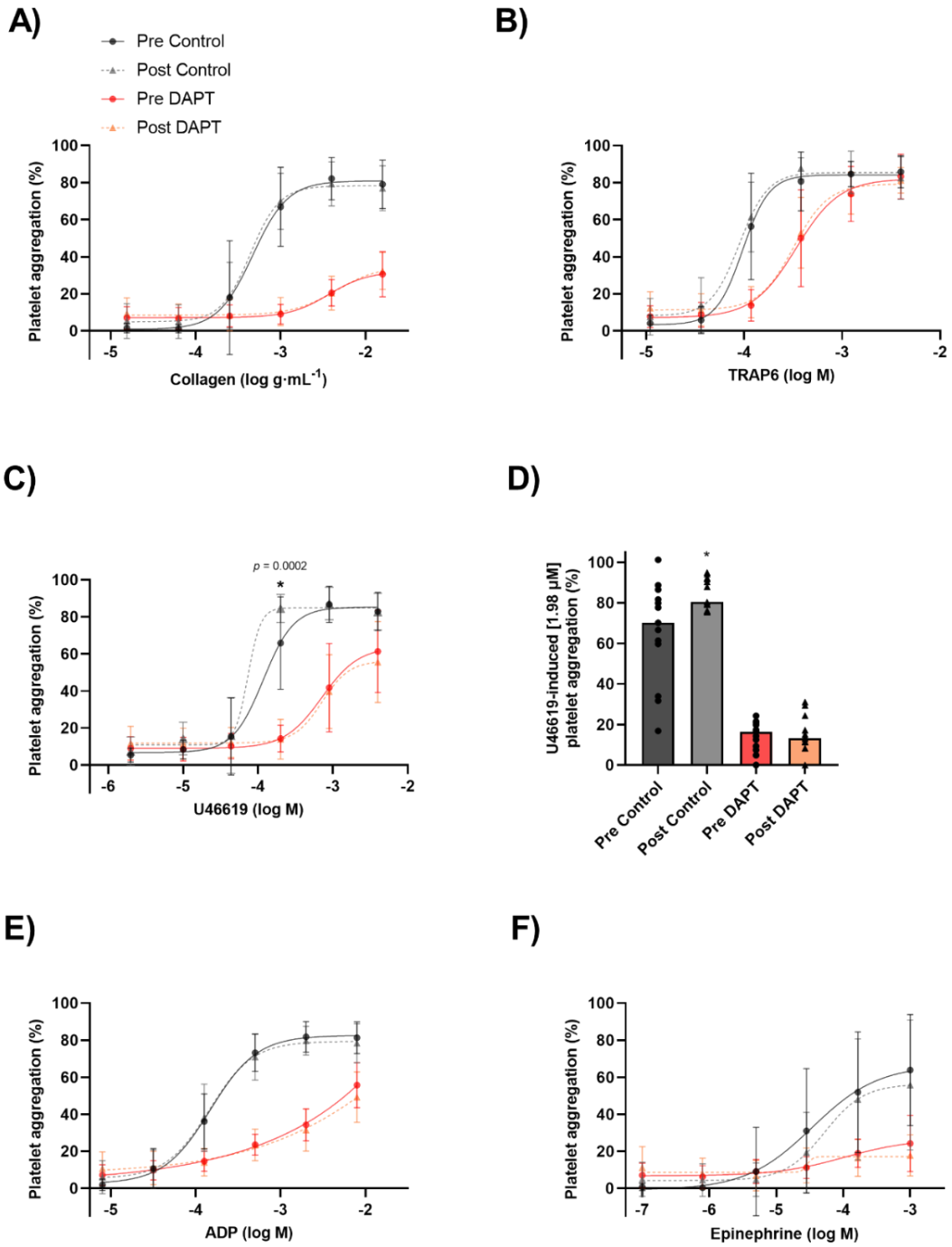

Figure 4-5: Basal platelet reactivity.

Basal platelet reactivity (expressed as platelet aggregation (%)) to 6 concentrations of the platelet agonists before and after 8 weeks of exercise training with and without dual anti-platelet therapy (DAPT). (**A**) collagen, (**B**) thrombin receptor activator peptide 6 (TRAP6), (**C**) U46619 (U4), (**D**) U46619-induced aggregation at [1.98 µM], (**E**) adenosine 5'-diphosphate (ADP), (**F**) epinephrine. *, indicates a statistically significant increase compared to pre-training (n=13 for all agonists).

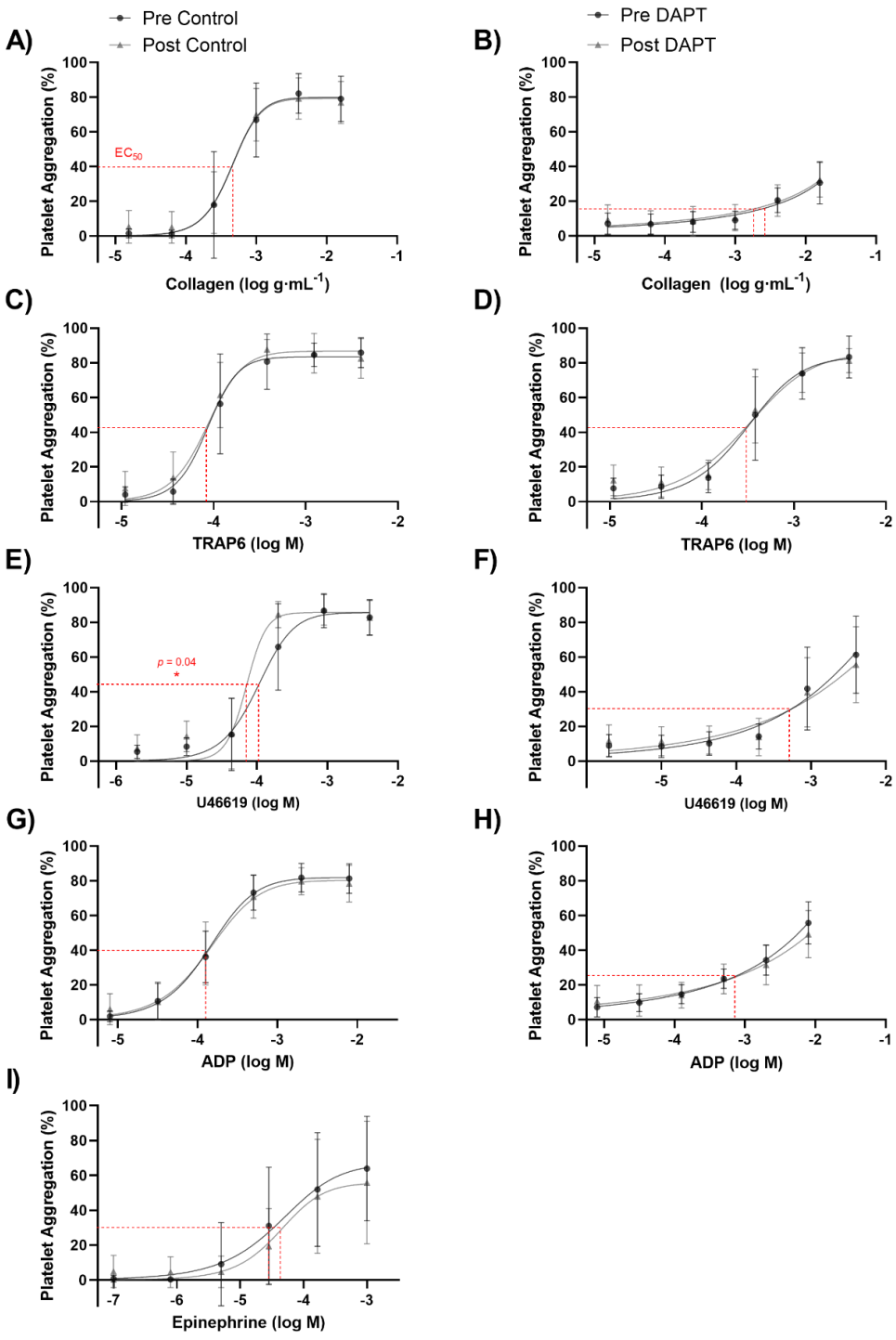

Figure 4-6: Half maximal effective concentration (EC_{50}) for platelet aggregation.

The half maximal effective concentration (EC_{50}) for platelet aggregation with (B, D, F, H) or without (A, C, E, G, I) dual anti-platelet therapy (DAPT). (**A**) collagen, (**B**) collagen with DAPT, (**C**) thrombin receptor activator peptide 6 (TRAP6), (**D**) TRAP6 with DAPT, (**E**) U46619, (**F**) U46619 with DAPT, (**G**) ADP, (**H**) ADP with DAPT, and (**I**) epinephrine. *, indicates a statistically significant decrease with training (n=13 for all agonists).

4.4.4 Exercise Training, Dual Anti-Platelet Therapy, and Basal Platelet Reactivity

There was a main effect of condition, whereby DAPT significantly inhibited collagen-, TRAP6-, ADP-, U46619-, and epinephrine-induced platelet aggregation ($p < 0.0001$ for all agonists). However, 8 weeks of exercise training did not influence the basal platelet reactivity response or EC_{50} to collagen, TRAP6, ADP, U46619, or epinephrine in the presence of DAPT (Figures 4-5 and 4-6). An EC_{50} for epinephrine-induced platelet aggregation in the presence of DAPT could not be generated.

4.4.5 Exercise Training and Basal Platelet Prostacyclin Sensitivity

Prostacyclin sensitivity was evaluated as the inhibition of aggregation to a known concentration of platelet agonist as well as the concentration of prostacyclin required to elicit half of the maximal inhibition of platelet aggregation (IC_{50}). Maximal platelet aggregation in the presence of vehicle treatment was not different for any agonist pre- versus post-training. Increasing concentrations of prostacyclin resulted in greater inhibition of platelet aggregation ($p < 0.0001$ for all agonists). Prostacyclin sensitivity was improved after training, as evidenced by a ~29% greater inhibition of collagen-induced platelet aggregation (10 $\mu g \cdot mL^{-1}$) in the presence of 10 nM prostacyclin (40.9 ± 23.5 vs. 52.9 ± 13.5% inhibition of aggregation; $p = 0.01$) after, compared to before, training (Figure 4-7A) (no change IC_{50}; $p = 0.27$; Figure 4-8A). Thromboxane A_2 mimetic U46619 [10 µM]-induced platelet aggregation was inhibited to a greater extent (~99%) after training in the presence of [1 nM] prostacyclin (24.7 ± 18.1 vs. 49.1 ± 11.5% inhibition of aggregation; $p < 0.0001$) compared to before training (Figure 4-7C) (lower IC_{50}; $p = 0.01$; Figure 4-8E). Lastly, training led to a further inhibition (~44%) of [20 µM] ADP-induced

platelet aggregation in the presence of [30 nM] prostacyclin after training (27.5 ± 15.3 vs. 39.6 ± 15.9% inhibition of aggregation; p = 0.002) compared to before training (Figure 4-7D) (lower IC_{50}; p = 0.001; Figure 4-8G). Exercise training did not significantly alter the inhibitory effects of prostacyclin on TRAP6-induced platelet aggregation (lower IC_{50}; p = 0.03; Figure 4-8C).

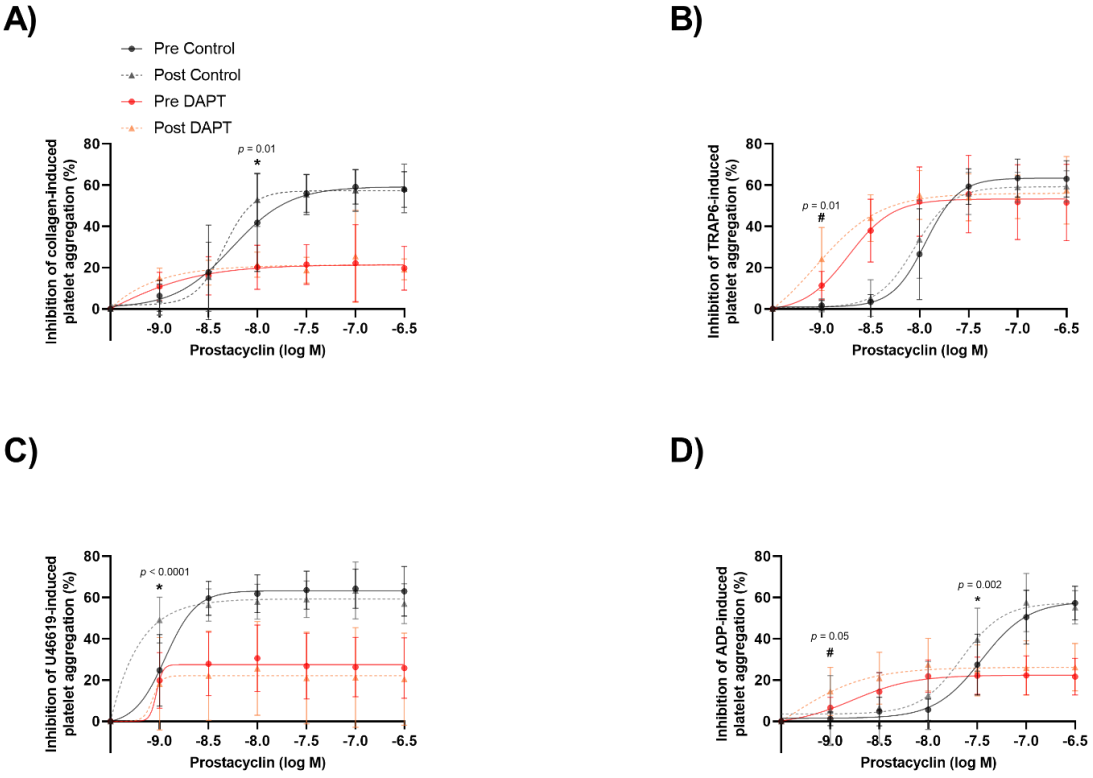

Figure 4-7: Platelet sensitivity to the anti-aggregatory effects of prostacyclin [1 – 300 nM], with and without dual anti-platelet therapy (DAPT).

Agonist-induced platelet aggregation to (**A**) 10 µg·mL^{-1} collagen (n=12), (**B**) 10 µM thrombin receptor activator peptide 6 (TRAP6) (n=13), (**C**) 10 µM U46619 (n=12), and (**D**) 20 µM adenosine 5'-diphosphate (ADP). *, indicates a statistically significant decrease with training with the control treatment. #, indicates a statistically significant decrease with training with the DAPT treatment.

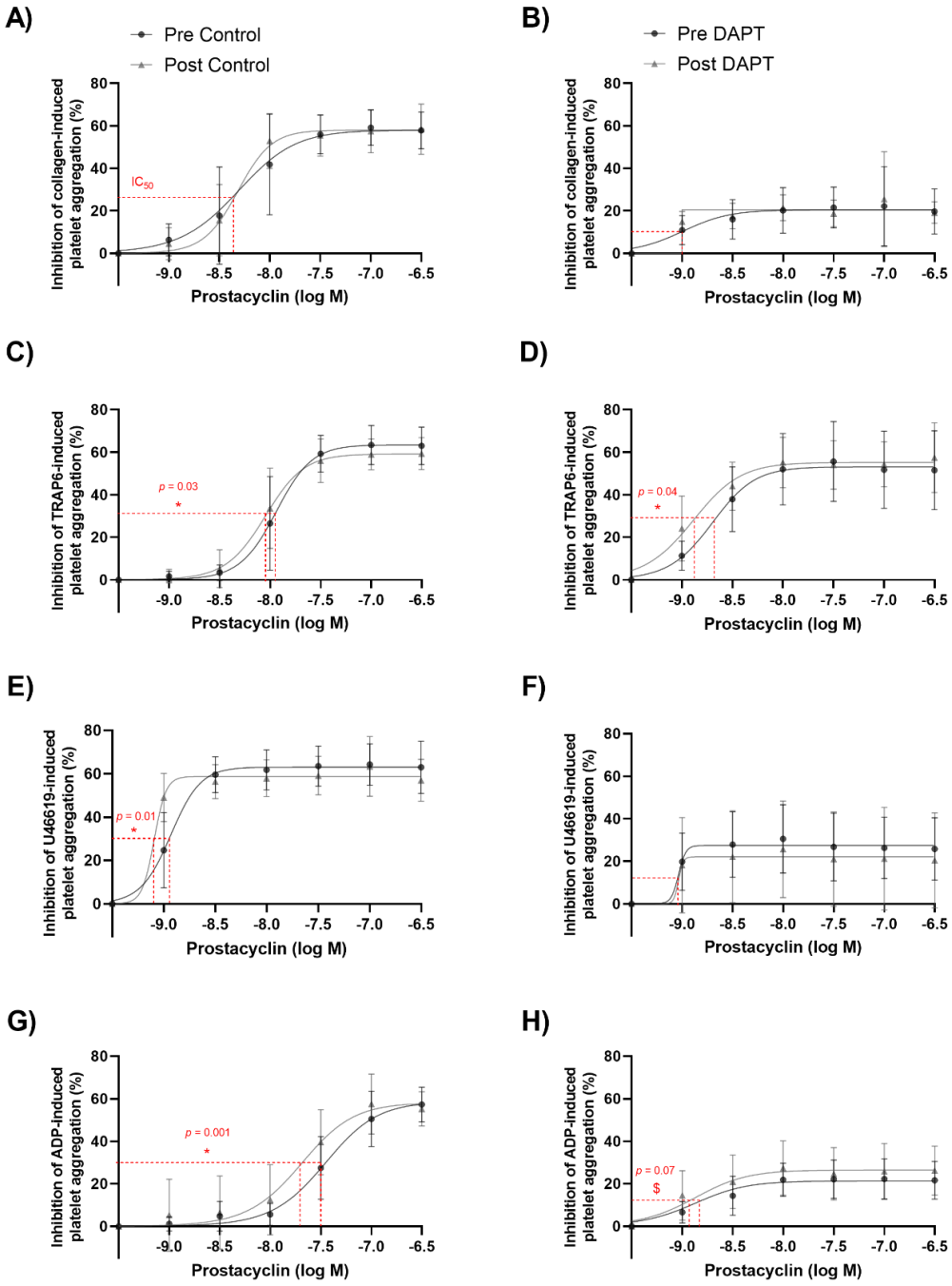

Figure 4-8: Half maximal inhibitory concentration (IC50) for inhibition of platelet aggregation by prostacyclin.

The half maximal inhibitory concentration (IC_{50}) for inhibition of platelet aggregation by prostacyclin [1 – 300 nM] with (B, D, F, H) or without (A, C, E, G) dual anti-platelet therapy (DAPT). (**A**) collagen (n=12), (**B**) collagen with DAPT (n=12), (**C**) thrombin receptor activator peptide 6 (TRAP6; n=13), (**D**) TRAP6 with DAPT (n=13), (**E**) U46619 (n=12), (**F**) U46619 with DAPT (n=12), (**G**) adenosine 5'-diphosphate (ADP) (n=12), and (**H**) ADP with DAPT (n=12). *, indicates a statistically significant decrease with training. $, indicates a trend for a decrease with training.

4.4.6 Exercise Training and Prostacyclin Sensitivity in the Presence of DAPT

Maximal platelet aggregation in the presence of DAPT treatment was not different for any agonist pre- versus post-training. DAPT elicited a main effect of condition by further facilitating prostacyclin-induced inhibited of collagen-, TRAP6-, ADP-, U46619-, and epinephrine-induced platelet aggregation ($p < 0.0001$ for all agonists). The exercise training program potentiated the inhibitory effects of DAPT in the context of prostacyclin sensitivity; after training, [10 μM] TRAP6-induced platelet aggregation was inhibited to a greater extent (~113%) in the presence of [1 nM] prostacyclin (11.3 ± 7.1 vs. 24.1 ± 15.9% inhibition of aggregation; p = 0.01) compared to before training (Figure 4-7B) (lower IC_{50}; $p = 0.04$; Figure 4-8D). Moreover, the training program resulted in [20 μM] ADP-induced platelet aggregation being further inhibited (~120%) by [1 nM] prostacyclin (6.6 ± 5.0 vs. 14.5 ± 12.2% inhibition of aggregation; $p = 0.05$) compared to before training (Figure 4-7D) (trend for lower IC_{50}; $p = 0.07$; Figure 4-8H). There was no effect of training on the inhibition of platelet aggregation to collagen (Figures 4-7A and 4-8B) or U46619 (Figures 4-7C and 4-8F) in the presence of DAPT.

4.4.7 Protein Expression in Isolated Platelets

The exercise training program did not affect the protein expression of thromboxane A_2 receptor ($p = 0.83$; Figure 4-9A), thromboxane A_2 synthase ($p = 0.67$; Figure 4-9B), cyclooxygenase-1 ($p = 0.52$; Figure 4-9C), or prostacyclin receptor ($p = 0.30$; Figure 4-9D) in isolated platelets. Representative blots are shown (Figure 4-9E).

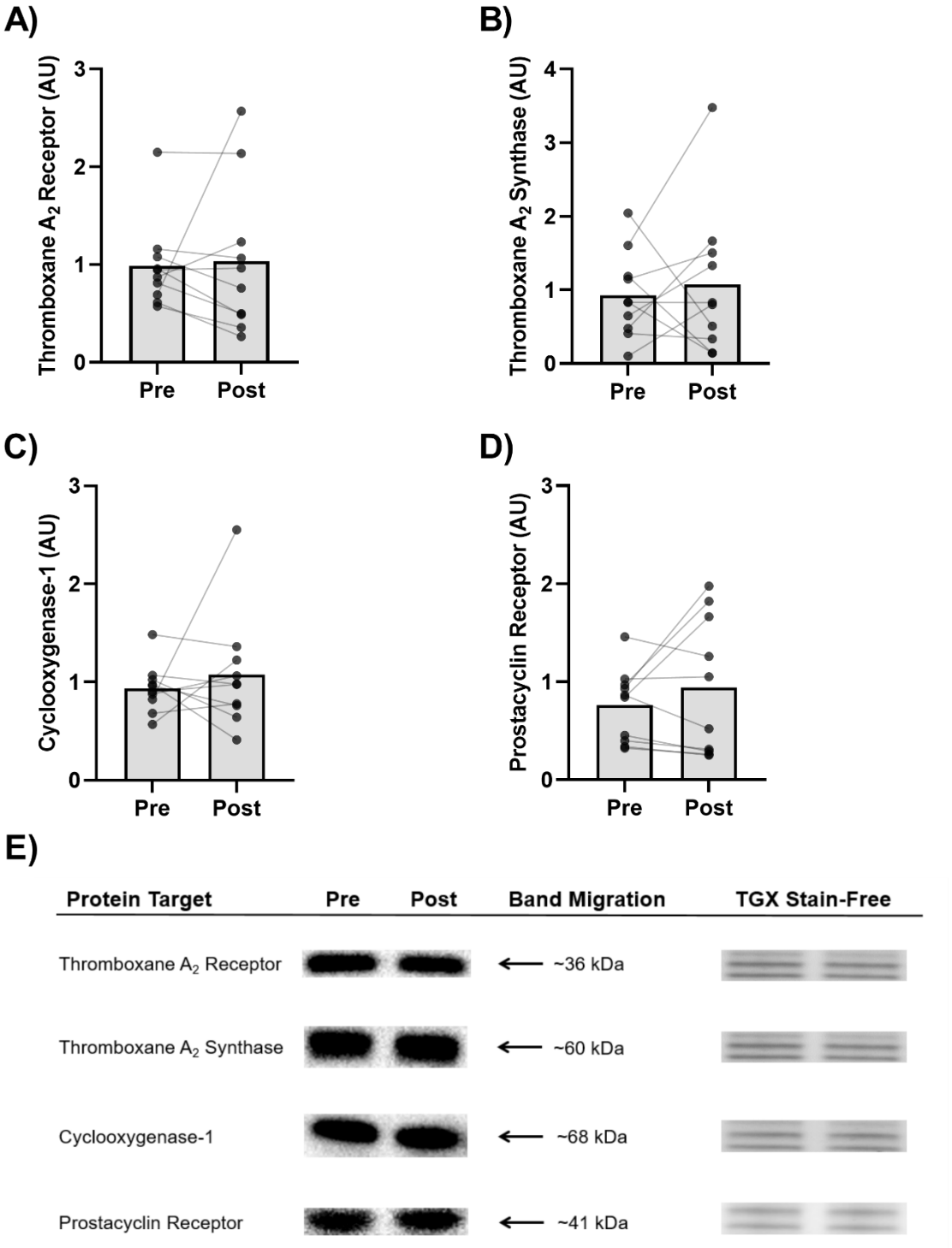

*Figure 4-9: Western blots on platelets isolated from whole blood (*n=*10 for all proteins).*

(**A**) Thromboxane A_2 Synthase (p = 0.829), (**B**) Thromboxane A_2 Receptor (p = 0.686), (**C**) Cyclooxygenase-1 (p = 0.844). (**D**) Prostacyclin Receptor (p = 0.04), and (**E**) Representative western blots and TGX Stain-Free image. Protein content is expressed in arbitrary units (AU).

4.4.8 Plasma 6-keto $PGF_{1\alpha}$ and Thromboxane B_2 Levels

Circulating resting 6-keto $PGF_{1\alpha}$ levels were unaltered by 8 weeks of exercise training (73.3 ± 37.5 pg·mL^{-1}) compared to pre-training (71.5 ± 29.1 pg·mL^{-1}) (p = 0.90; Figure 4-10A). Similarly, circulating resting thromboxane B_2 levels were unchanged after the training intervention (4.62 ± 9.11 ng·mL^{-1}) compared to pre-training (4.74 ± 9.11 ng·mL^{-1}) (p = 0.58; Figure 4-10B). Lastly, there was no difference in the resting plasma 6-keto $PGF_{1\alpha}$ to thromboxane B_2 ratio before (0.06 ± 0.06 pg·mL^{-1}) and after (0.05 ± 0.03 pg·mL^{-1}) the training intervention (p = 0.47).

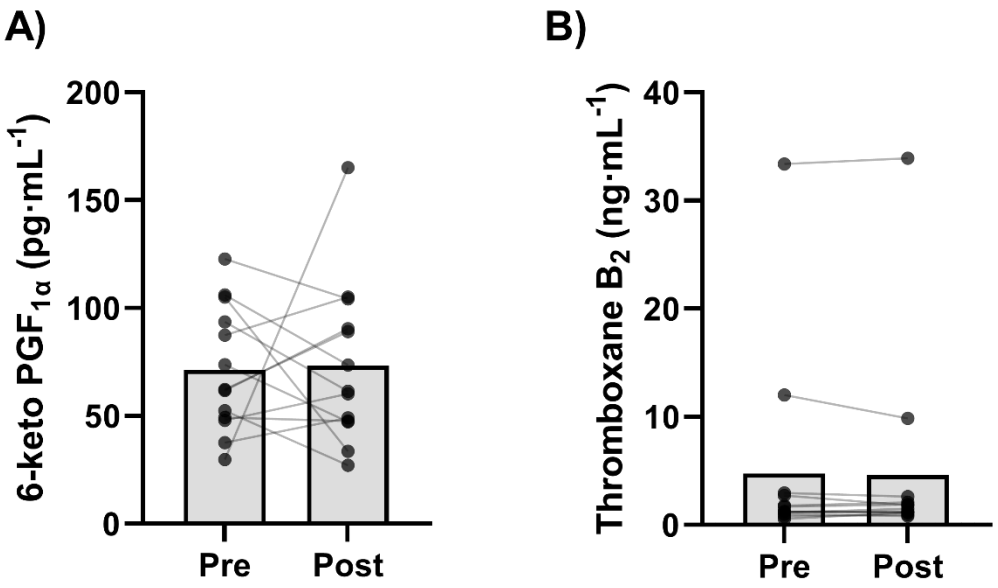

*Figure 4-10: Plasma levels of (**A**) 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) (p = 0.895) and (**B**) thromboxane B_2 (p = 0.579) measured pre- and post-training (n=13).*

4.5 Discussion

The primary findings in this study were that 8 weeks of high-intensity exercise training: **(1)** increased platelet sensitivity to the inhibitory effects of prostacyclin to collagen-, U46619-, and ADP-induced aggregation; **(2)** potentiated the effect of DAPT on prostacyclin sensitivity to TRAP6- and ADP-induced aggregation; and **(3)** did not influence platelet protein expression of thromboxane A_2 receptor, thromboxane A_2 synthase, cyclooxygenase-1, or prostacyclin receptor.

4.5.1 Exercise Training Improves Basal Prostacyclin Sensitivity

Prostacyclin is a potent inhibitor of platelet aggregation, but only a paucity of studies have examined the role of exercise training on platelet prostacyclin sensitivity and only one study has examined it in postmenopausal women [9]. Our findings are in agreement with previous evidence, while significantly expanding the depth of the literature regarding the underlying mechanisms of action. Similar to Lundberg Slingsby et al. [9], we showed an improved platelet prostacyclin sensitivity to collagen-induced aggregation following 8 weeks of high-intensity training in postmenopausal women (Figure 4-7A). Interestingly, the participants in the previous investigation were ~3 years postmenopausal, whereas the women in this study were, on average, ~10 years postmenopausal, and a growing body of evidence suggests that cardiovascular adaptations to exercise training may be more difficult to accrue with an increasing number of years after menopause [31]. Accordingly, our novel findings are valuable, because we demonstrate that prostacyclin sensitivity can still be augmented by as little as 8 weeks of exercise training in women that are ~10 years postmenopausal, despite noted difficulty in obtaining other cardiovascular adaptations.

Furthering this point, we expanded on the existing evidence by also showing improvements in platelet prostacyclin sensitivity to TRAP6-, and ADP-induced aggregation after training in postmenopausal women (Figures 4-7C, 4-7D). Moreover, we demonstrate that, after training, lower concentrations of prostacyclin are required to elicit 50% inhibition of maximal platelet aggregation in response to TRAP6 (Figure 4-8C), U46619 (Figure 4-8E), and ADP (Figure 4-8G), providing compelling support that prostacyclin sensitivity is improved via multiple platelet pathways following exercise training. To explore the possible mechanism underpinning the improved sensitivity, we determined the protein expression of the prostacyclin receptor in isolated platelets. Exercise training did not change the receptor expression (Figure 4-9D), suggesting that the improvement in prostacyclin sensitivity was mediated by an improvement in receptor sensitivity or downstream signalling pathways (e.g., adenylate cyclase or cyclic adenosine monophosphate), rather than receptor quantity.

To understand if training also influenced circulating levels of prostacyclin in the women, we assessed the stable prostacyclin biomarker 6-keto $PGF_{1\alpha}$ before and after the training intervention. In contrast to our initial hypothesis and previous observations [32,33], resting plasma prostacyclin levels were similar before and after training (Figure 4-10A). However, the previous training intervention showing an increase in systemic plasma prostacyclin levels was in young healthy individuals and 6 months in duration [33]. Therefore, it is plausible that a longer intervention is required to elicit this systemic elevation, particularly in postmenopausal women. In line with this notion, Gliemann et al.

[32] only showed increased local prostacyclin release with acetylcholine infusion with 3 months of training in postmenopausal women.

4.5.2 Exercise Training Potentiates the Effects of DAPT on Prostacyclin Sensitivity

A main goal of this study was to assess the potential synergism between exercise training and DAPT on prostacyclin sensitivity. Previous evidence suggests that the efficacy of DAPT, and specifically $P2Y_{12}$ inhibitors, is highly inter-individualized [34]. However, there is a significant positive association between an individual's vascular health and the efficacy of DAPT treatment, whereby individuals with greater vascular health are more sensitive to DAPT, since $P2Y_{12}$ inhibitors potentiate the anti-platelet effects of prostacyclin and NO [14–16]. This led us to hypothesize that exercise training, which has been shown to increase NO [19–21] and prostacyclin [32,33] bioavailability as well as prostacyclin sensitivity [9,22], could enhance the inhibitory effects of prostacyclin in the presence of DAPT.

In line with our initial hypothesis, we observed that as little as 8 weeks of exercise training significantly potentiated the response to DAPT in the context of prostacyclin sensitivity. Specifically, we found that after training, the inhibitory effects of DAPT on TRAP6 and ADP-induced platelet aggregation were enhanced when exposed to low concentrations of prostacyclin, an endogenous platelet inhibitor (Figures 4-7B, 4-7D). Additionally, after training, we observed a significant lowering of the IC_{50} for prostacyclin-induced inhibition of platelet aggregation to TRAP6 (Figure 4-8D) as well as a trend for a lowering of the IC_{50} for prostacyclin-induced inhibition of platelet aggregation to ADP (Figure 4-8H). These findings are the first in women, but are in line with those of a cross-sectional study in men

of differing lifelong physical activity levels [22]. These findings, in conjunction with the previous observation of DAPT (specifically $P2Y_{12}$ receptor antagonists) enhancing prostacyclin sensitivity [35] and our current observation of no change in prostacyclin receptor protein expression (Figure 4-9D), suggest that exercise training may enhance the efficacy of DAPT on prostacyclin receptor function or downstream signalling pathways (e.g., adenylate cyclase or cyclic adenosine monophosphate).

Taken together, these findings provide novel evidence that postmenopausal women who perform regular exercise training may have greater prostacyclin sensitivity with and without DAPT compared to sedentary postmenopausal women. Accordingly, less DAPT medication may be required for these individuals and personalized dosing schemes could be considered to minimize the problematic side effects of these drugs in terms of bleeding risk [22,23]. Moreover, this data supports the notion that regular physical activity is an important tool for promoting anti-thrombotic protection in previously sedentary post-menopausal women.

4.5.3 Exercise Training Increases Basal Platelet Sensitivity to Thromboxane-Induced Aggregation

A scarcity of data exists regarding the effects of exercise training on basal platelet responses in postmenopausal women [9,36]. These authors showed a reduction in platelet reactivity and a concomitant rightward shift in the platelet aggregation curve to several platelet agonists, including thromboxane, in exercise trained individuals [9,22]. Collectively, these findings reflect reduced platelet sensitivity, and accordingly our contrasting findings were unexpected, as exercise training increased basal platelet

aggregation in response to the thromboxane receptor analogue U46619 (Figures 4-5C, 4-5D). Specifically, the increased platelet reactivity to the same concentration of U46619 ([1.98 µM]) after training may suggest increased sensitivity to this thromboxane receptor analogue. In conjunction with this observation, we report a significant lowering of the EC50 (Figure 4-6E), further demonstrating a sensitization of the platelet aggregation curve to U46619 following 8 weeks of training. Combined, these findings suggest that less thromboxane is required to elicit platelet aggregation following exercise training. However, it is worthwhile to consider that previous findings were in healthy men [22] and premenopausal women [9], and it is possible that the adaptations to exercise training are divergent in postmenopausal women lacking endogenous estrogen. Notably, Lundberg Slingsby et al. [9] reported no effect of 12 weeks of aerobic exercise training on basal platelet reactivity in early postmenopausal women. Although, the parameter(s) driving these divergent findings is currently unclear and warrants future investigations, it is possible that menopause and the time after menopause influences the platelet adaptations to exercise training. This may further explain the divergence between our findings in women ~10 years postmenopausal and in the previous study where the women were ~3 years postmenopausal [9]. Moreover, it is important to consider this finding in conjunction with the observed increase in inhibition of U46619-induced aggregation in the presence of prostacyclin following exercise training, where the total *in-vivo* platelet response may favour inhibition of aggregation due to the continuous release of prostacyclin from the endothelium. Nevertheless, to understand the mechanism(s) underpinning the increased sensitivity observed in this study, we explored

various components of the thromboxane pathway for platelet activation. However, we did not observe any significant changes in the parameters we measured. Specifically, there were no changes in protein expression of the thromboxane A_2 receptor in isolated platelets following exercise training, suggesting that increased platelet reactivity was not due to an increase in thromboxane receptor content (Figure 4-9A). Moreover, we did not observe a change in resting plasma thromboxane B_2 levels (Figure 4-10B), or in platelet protein expression of cyclooxygenase-1 or thromboxane A_2 synthase, enzymes critical for platelet production of thromboxane A_2 (Figures 4-9B, 4-9C). Interestingly, previous investigations have demonstrated significant reductions in plasma thromboxane B_2 levels following 16 weeks [33] or 6 months [37] of exercise training, indicating a longer intervention may be necessary for this adaptation.

4.5.4 Exercise Training Does Not Influence Basal Platelet Reactivity with DAPT

Contrary to our initial hypothesis, we did not show any changes in the basal platelet reactivity response to DAPT following the training period (Figures 4-5 and 4-6). Our hypothesis was based on the findings from a previous cross-sectional study [22], which showed that lifelong trained middle-aged men present greater basal sensitivity to pharmacological inhibition by DAPT compared to untrained and moderately trained men. However, a lifetime of exercise training elicits numerous cardioprotective effects, making it difficult to compare to 8 weeks of training in previously sedentary postmenopausal women. Accordingly, future studies with longer interventions are required to verify whether exercise training can act synergistically with DAPT on basal platelet reactivity in postmenopausal women. Moreover, this study should be performed in previously

sedentary older men to determine the reversibility of inactive aging on platelet function and the responses to DAPT in this cohort.

4.5.5 Study Limitations

DAPT is not a conventional anti-thrombotic therapy for postmenopausal women and is normally only prescribed to prevent further cardiovascular events. Notably, individuals that receive DAPT tend to have additional comorbidities, such as arterial hypertension, diabetes, or obesity. It is therefore important to point out that this study was performed in healthy but sedentary postmenopausal women and additional studies are required to elucidate the effects in postmenopausal women at risk of cardiovascular events. DAPT treatment was administered acutely *in-vitro*, which may produce divergent results than *in-vivo* oral administration due to different pharmacokinetic profiles. Furthermore, the women who participated in this study were on average ~10 years postmenopausal. Recent evidence suggests that cardiovascular adaptations to exercise training may be more difficult to achieve in this cohort, which may explain our lack of improvement in $\dot{V}O_{2max}$ [31]. Moreover, and perhaps more importantly, a longer training intervention may have produced more dramatic effects on the platelet responses. Accordingly, a greater depth of research is required to determine the optimal training modality and duration for improving platelet function in postmenopausal women as well as whether the number of years after menopause influences the platelet adaptive response to exercise training.

4.6 Conclusions

This study demonstrates that a rather brief period (8 weeks) of high-intensity exercise training is sufficient to induce significant improvements in platelet sensitivity to prostacyclin with and without DAPT in previously sedentary postmenopausal women. This effect may be mediated by improvements in platelet prostacyclin receptor function or downstream signalling pathways (e.g., adenylate cyclase or cyclic adenosine monophosphate), as we did not observe training-induced changes in prostacyclin receptor protein expression or systemic plasma 6-keto $PGF_{1\alpha}$. These findings emphasize the importance of promoting physical activity as a tool for reducing thrombotic risk in postmenopausal women and given the enhanced prostacyclin sensitivity and DAPT enhanced prostacyclin sensitivity after training, healthcare providers may consider training status when prescribing DAPT to this cohort.

4.6.1 Perspectives

There has been increasing awareness around the underrepresentation of women in pharmaceutical research and the notion that the responses to therapeutic interventions are divergent between men and women [38]. Accordingly, there has been a recent and critical push for the expansion of female inclusion in clinical decision making to maximize the efficacy and safety of pharmaceutical treatments across the sexes [39]. Notably, this gap extends to DAPT research initiatives [23,24]. The existing literature suggests that after aspirin therapy, women maintain higher platelet reactivity, highlighting a lesser benefit of the treatment [25]. Similar findings have been reported for the $P2Y_{12}$ inhibitor clopidogrel [26]. These data highlight the importance of including postmenopausal

women in DAPT research, and this study has taken a step in the right direction for promoting exercise as an additional beneficial therapy for improving platelet function in this cohort. However, the call for inclusion of women in pharmacological and exercise therapy studies persists and a greater depth of research is required to provide the best individualized care for patients.

4.7 References

1. Alkarithi, G.; Duval, C.; Shi, Y.; Macrae, F.L.; Ariëns, R.A.S. Thrombus Structural Composition in Cardiovascular Disease. Arteriosclerosis, Thrombosis, and Vascular Biology 2021, 41, 2370–2383, doi:10.1161/ATVBAHA.120.315754.

2. Engbers, M.J.; Van Hylckama Vlieg, A.; Rosendaal, F.R. Venous Thrombosis in the Elderly: Incidence, Risk Factors and Risk Groups. Journal of Thrombosis and Haemostasis 2010, 8, 2105–2112, doi:10.1111/j.1538-7836.2010.03986.x.

3. Troy, G.C. An Overview of Hemostasis. Veterinary Clinics of North America: Small Animal Practice 1988, 18, 5–20, doi:10.1016/S0195-5616(88)50003-7.

4. Bucciarelli, P.; Mannucci, P.M. The Hemostatic System through Aging and Menopause. Climacteric 2009, 12 Suppl 1, 47–51, doi:10.1080/13697130903006365.

5. Kurachi, K.; Kurachi, S. Genetic Mechanisms of Age Regulation of Blood Coagulation. Arteriosclerosis, Thrombosis, and Vascular Biology 2000, 20, 902–906, doi:10.1161/01.ATV.20.4.902.

6. Le Blanc, J.; Lordkipanidzé, M. Platelet Function in Aging. Front Cardiovasc Med 2019, 6, 109, doi:10.3389/fcvm.2019.00109.

7. Meade, T.W.; Vickers, M.V.; Thompson, S.G.; Stirling, Y.; Haines, A.P.; Miller, G.J. Epidemiological Characteristics of Platelet Aggregability. Br Med J (Clin Res Ed) 1985, 290, 428–432, doi:10.1136/bmj.290.6466.428.

8. Butkiewicz, A.M.; Kemona, H.; Dymicka-Piekarska, V.; Matowicka-Karna, J. [Does menopause affect thrombocytopoiesis and platelet activation?]. Przegl Lek 2006, 63, 1291–1293.

9. Lundberg Slingsby, M.H.; Nyberg, M.; Egelund, J.; Mandrup, C.M.; Frikke-Schmidt, R.; Kirkby, N.S.; Hellsten, Y. Aerobic Exercise Training Lowers Platelet Reactivity and Improves Platelet Sensitivity to Prostacyclin in Pre- and Postmenopausal Women. J Thromb Haemost 2017, 15, 2419–2431, doi:10.1111/jth.13866.

10. Roshan, T.M.; Normah, J.; Rehman, A.; Naing, L. Effect of Menopause on Platelet Activation Markers Determined by Flow Cytometry. American Journal of Hematology 2005, 80, 257–261, doi:10.1002/ajh.20472.

11. Nakano, Y.; Oshima, T.; Matsuura, H.; Kajiyama, G.; Kambe, M. Effect of 17β-Estradiol on Inhibition of Platelet Aggregation In Vitro Is Mediated by an Increase in NO Synthesis. Arteriosclerosis, Thrombosis, and Vascular Biology 1998, 18, 961–967, doi:10.1161/01.ATV.18.6.961.

12. Sobrino, A.; Oviedo, P.J.; Novella, S.; Laguna-Fernandez, A.; Bueno, C.; García-Pérez, M.A.; Tarín, J.J.; Cano, A.; Hermenegildo, C. Estradiol Selectively Stimulates

Endothelial Prostacyclin Production through Estrogen Receptor-α. Journal of Molecular Endocrinology 2010, 44, 237–246, doi:10.1677/JME-09-0112.

13. Collet, J.-P.; Thiele, H.; Barbato, E.; Barthélémy, O.; Bauersachs, J.; Bhatt, D.L.; Dendale, P.; Dorobantu, M.; Edvardsen, T.; Folliguet, T.; et al. 2020 ESC Guidelines for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation. European Heart Journal 2021, 42, 1289–1367, doi:10.1093/eurheartj/ehaa575.

14. Chan, M.V.; Knowles, R.B.M.; Lundberg, M.H.; Tucker, A.T.; Mohamed, N.A.; Kirkby, N.S.; Armstrong, P.C.J.; Mitchell, J.A.; Warner, T.D. P2Y12 Receptor Blockade Synergizes Strongly with Nitric Oxide and Prostacyclin to Inhibit Platelet Activation. Br J Clin Pharmacol 2016, 81, 621–633, doi:10.1111/bcp.12826.

15. Kirkby, N.S.; Lundberg, M.H.; Chan, M.V.; Vojnovic, I.; Solomon, A.B.; Emerson, M.; Mitchell, J.A.; Warner, T.D. Blockade of the Purinergic P2Y12 Receptor Greatly Increases the Platelet Inhibitory Actions of Nitric Oxide. Proc Natl Acad Sci U S A 2013, 110, 15782–15787, doi:10.1073/pnas.1218880110.

16. Warner, T.D.; Armstrong, P.C.; Chan, M.V.; Knowles, R.B. The Importance of Endothelium-Derived Mediators to the Efficacy of Dual Anti-Platelet Therapy. Expert Rev Hematol 2016, 9, 223–225, doi:10.1586/17474086.2016.1140035.

17. Candelaria, D.; Randall, S.; Ladak, L.; Gallagher, R. Health-Related Quality of Life and Exercise-Based Cardiac Rehabilitation in Contemporary Acute Coronary Syndrome Patients: A Systematic Review and Meta-Analysis. Qual Life Res 2020, 29, 579–592, doi:10.1007/s11136-019-02338-y.

18. Green, D.J.; Smith, K.J. Effects of Exercise on Vascular Function, Structure, and Health in Humans. Cold Spring Harb Perspect Med 2018, 8, a029819, doi:10.1101/cshperspect.a029819.

19. Braga, V.A.V.N.; Couto, G.K.; Lazzarin, M.C.; Rossoni, L.V.; Medeiros, A. Aerobic Exercise Training Prevents the Onset of Endothelial Dysfunction via Increased Nitric Oxide Bioavailability and Reduced Reactive Oxygen Species in an Experimental Model of Menopause. PLoS One 2015, 10, e0125388, doi:10.1371/journal.pone.0125388.

20. Seals, D.R.; Nagy, E.E.; Moreau, K.L. Aerobic Exercise Training and Vascular Function with Ageing in Healthy Men and Women. The Journal of Physiology 2019, 597, 4901–4914, doi:10.1113/JP277764.

21. Zaros, P.R.; Pires, C.E.R.; Bacci, M.; Moraes, C.; Zanesco, A. Effect of 6-Months of Physical Exercise on the Nitrate/Nitrite Levels in Hypertensive Postmenopausal Women. BMC Women's Health 2009, 9, 17, doi:10.1186/1472-6874-9-17.

22. Lundberg Slingsby, M.H.; Gliemann, L.; Thrane, M.; Rytter, N.; Egelund, J.; Chan, M.V.; Armstrong, P.C.; Warner, T.D.; Hellsten, Y. Platelet Responses to Pharmacological

and Physiological Interventions in Middle-Aged Men with Different Habitual Physical Activity Levels. Acta Physiol 2018, 223, e13028, doi:10.1111/apha.13028.

23. Pelliccia, F.; Gragnano, F.; Pasceri, V.; Marazzi, G.; Cacciotti, L.; Placanica, A.; Niccoli, G.; Palmerini, T.; Speciale, G.; Granatelli, A.; et al. Gender-Related Differences in Changes of Estimated Bleeding Risk in Patients on Dual Antiplatelet Therapy: The RE-SCORE Multicenter Prospective Registry. Platelets 2022, 0, 1–9, doi:10.1080/09537104.2022.2102602.

24. Pradhan, A.; Vishwakarma, P.; Sethi, R. Changing Scenario in Management of Acute Coronary Syndromes in Females—Evidence from Recent Studies. Indian J Cardiovasc Dis Women WINCARS 2018, 03, 245–250, doi:10.1055/s-0039-1683942.

25. Becker, D.M.; Segal, J.; Vaidya, D.; Yanek, L.R.; Herrera-Galeano, J.E.; Bray, P.F.; Moy, T.F.; Becker, L.C.; Faraday, N. Sex Differences in Platelet Reactivity and Response to Low-Dose Aspirin Therapy. JAMA 2006, 295, 1420–1427, doi:10.1001/jama.295.12.1420.

26. Ranucci, M.; Aloisio, T.; Di Dedda, U.; Menicanti, L.; de Vincentiis, C.; Baryshnikova, E. Gender-Based Differences in Platelet Function and Platelet Reactivity to P2Y12 Inhibitors. PLoS One 2019, 14, e0225771, doi:10.1371/journal.pone.0225771.

27. Lordkipanidzé, M.; Lowe, G.C.; Kirkby, N.S.; Chan, M.V.; Lundberg, M.H.; Morgan, N.V.; Bem, D.; Nisar, S.P.; Leo, V.C.; Jones, M.L.; et al. Characterization of Multiple Platelet Activation Pathways in Patients with Bleeding as a High-Throughput Screening Option: Use of 96-Well Optimul Assay. Blood 2014, 123, e11–e22, doi:10.1182/blood-2013-08-520387.

28. Leadbeater, P.D.M.; Kirkby, N.S.; Thomas, S.; Dhanji, A.-R.; TUCKER, A.T.; Milne, G.L.; Mitchell, J.A.; Warner, T.D. Aspirin Has Little Additional Anti-Platelet Effect in Healthy Volunteers Receiving Prasugrel. J Thromb Haemost 2011, 9, 2050–2056, doi:10.1111/j.1538-7836.2011.04450.x.

29. Winter, M.; Schneeweiss, T.; Cremer, R.; Biesinger, B.; Hengstenberg, C.; Prüller, F.; Wallner, M.; Kolesnik, E.; von Lewinski, D.; Lang, I.M.; et al. Platelet Reactivity Patterns in Patients Treated with Dual Antiplatelet Therapy. Eur J Clin Invest 2019, 49, e13102, doi:10.1111/eci.13102.

30. Russo, I.; Traversa, M.; Bonomo, K.; De Salve, A.; Mattiello, L.; Del Mese, P.; Doronzo, G.; Cavalot, F.; Trovati, M.; Anfossi, G. In Central Obesity, Weight Loss Restores Platelet Sensitivity to Nitric Oxide and Prostacyclin. Obesity (Silver Spring) 2010, 18, 788–797, doi:10.1038/oby.2009.302.

31. Gliemann, L.; Hellsten, Y. The Exercise Timing Hypothesis: Can Exercise Training Compensate for the Reduction in Blood Vessel Function after Menopause If Timed Right? The Journal of Physiology 2019, 597, 4915–4925, doi:10.1113/JP277056.

32. Gliemann, L.; Tamariz-Ellemann, A.; Baasch-Skytte, T.; Ehlers, T.S.; Gunnarsson, T.P. Increased Prostacyclin Formation after High-Intensity Interval Training in Late

Postmenopausal Women. Eur J Appl Physiol 2020, 120, 1711–1720, doi:10.1007/s00421-020-04405-6.

33. Hansen, A.H.; Nyberg, M.; Bangsbo, J.; Saltin, B.; Hellsten, Y. Exercise Training Alters the Balance between Vasoactive Compounds in Skeletal Muscle of Individuals with Essential Hypertension. Hypertension 2011, 58, 943–949, doi:10.1161/HYPERTENSIONAHA.111.176529.

34. Gan, X.-D.; Wei, B.-Z.; Fang, D.; Fang, Q.; Li, K.-Y.; Ding, S.-L.-Y.; Peng, S.; Wan, J. Efficacy and Safety Analysis of New P2Y12 Inhibitors versus Clopidogrel in Patients with Percutaneous Coronary Intervention: A Meta-Analysis. Current Medical Research and Opinion 2015, 31, 2313–2323, doi:10.1185/03007995.2015.1098600.

35. Cattaneo, M.; Lecchi, A. Inhibition of the Platelet P2Y12 Receptor for Adenosine Diphosphate Potentiates the Antiplatelet Effect of Prostacyclin. J Thromb Haemost 2007, 5, 577–582, doi:10.1111/j.1538-7836.2007.02356.x.

36. Singla, A.; Bliden, K.P.; Jeong, Y.-H.; Abadilla, K.; Antonino, M.J.; Muse, W.C.; Mathew, D.P.; Bailon, O.; Tantry, U.S.; Gurbel, P.A. Platelet Reactivity and Thrombogenicity in Postmenopausal Women. Menopause 2013, 20, 57–63, doi:10.1097/gme.0b013e31825ebafd.

37. Medvedev, I.N.; Karpov, V.Y.; Eremin, M.V.; Boldov, A.S.; Shalupin, V.I.; Voronova, N.N.; Malyshev, A.V. The Functional Characteristics of the Organism of Physically Inactive Students Who Have Started Regular Physical Training. J Biochem Technol 2021, 12, 33–37, doi:10.51847/LdHG6l2l5q.

38. Madla, C.M.; Gavins, F.K.H.; Merchant, H.A.; Orlu, M.; Murdan, S.; Basit, A.W. Let's Talk about Sex: Differences in Drug Therapy in Males and Females. Advanced Drug Delivery Reviews 2021, 175, 113804, doi:10.1016/j.addr.2021.05.014.

39. Liu, K.A.; Mager, N.A.D. Women's Involvement in Clinical Trials: Historical Perspective and Future Implications. Pharm Pract (Granada) 2016, 14, 708, doi:10.18549/PharmPract.2016.01.708.

4.8 Research Program Progression

In *Chapter 4*, we confirmed that high-intensity exercise training is a valuable tool for improving vascular health in postmenopausal women. Specifically, we revealed that 8 weeks of high-intensity exercise training can improve basal platelet function via enhanced prostacyclin sensitivity and potentiation of the inhibitory response to DAPT. This evidence suggests that exercise training status may be important to consider when prescribing DAPT, and less medication may be required to elicit the same effects. Therefore, regular physical activity is a valuable modifiable lifestyle factor that can impact vascular health and drug responsiveness. However, it remained unclear whether this response would be observed across all postmenopausal women. Specifically, we were interested in ascertaining whether the years after menopause impacted the vascular adaptations to an exercise training program. Accordingly, in *Chapter 5*, we reviewed the existing literature exploring the effects of exercise training on vascular health in postmenopausal women, with an emphasis on the *exercise timing hypothesis*.

CHAPTER 5

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

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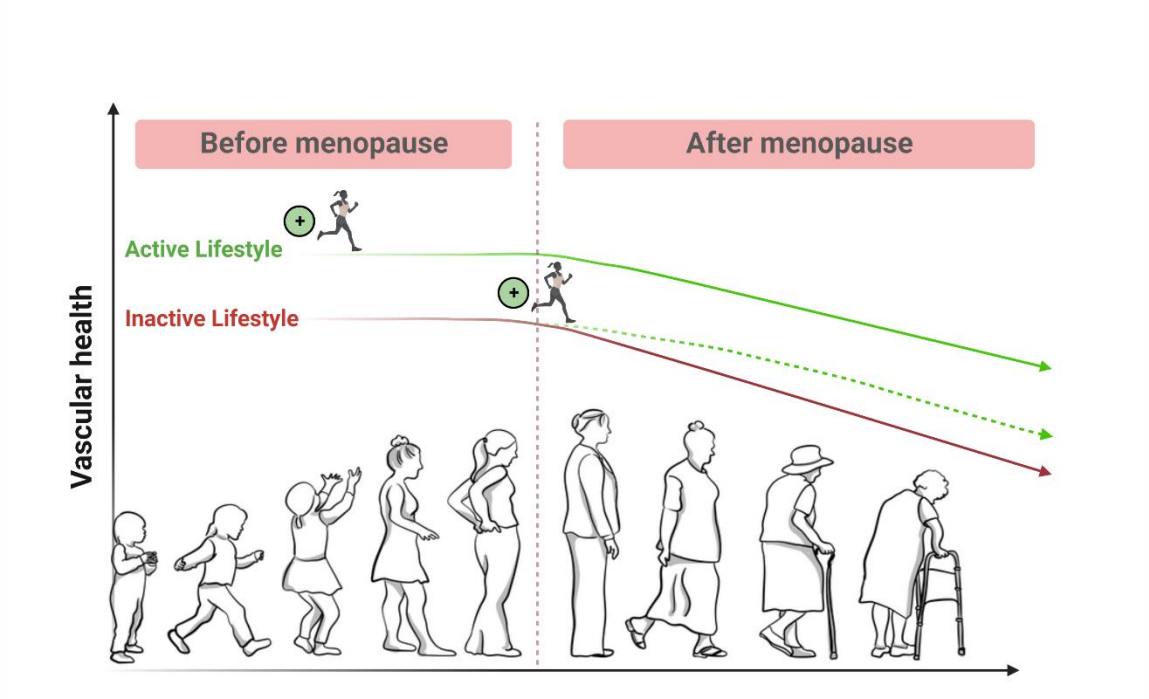

Figure 5-1: Review I graphical abstract.

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.

5.1 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 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.

5.2 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 ($\dot{V}O_{2max}$) decreases significantly with advancing age, where we have observed a significant negative linear

relationship between $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$ despite advancing age (Figure 5-2). 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).

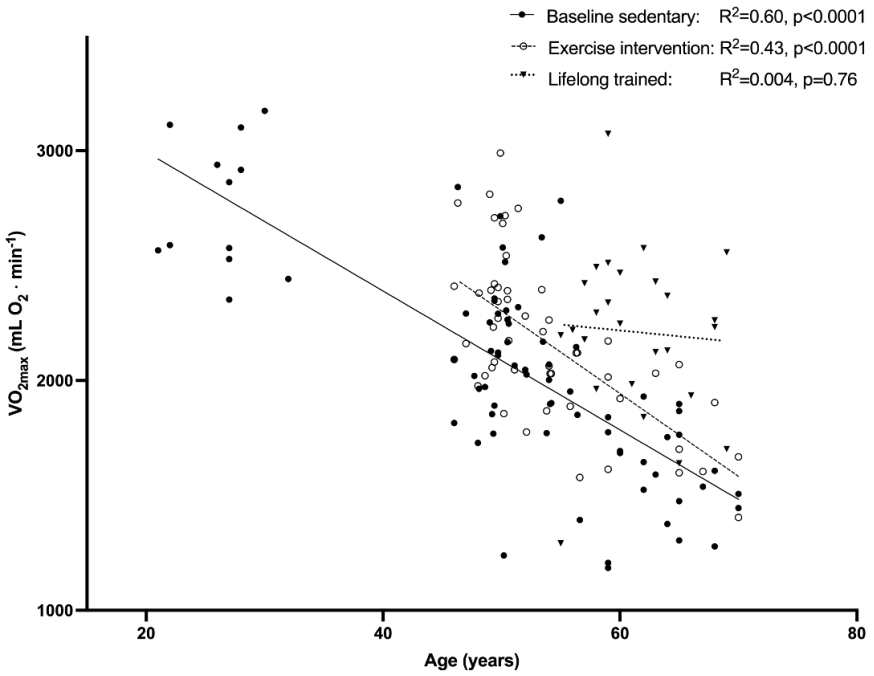

Figure 5-2: Relationship between age (years) and $\dot{V}O_{2max}$ (mL $O_2 \cdot min^{-1}$).

Linear correlations between $\dot{V}O_{2max}$ 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 of 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 $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$ 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.

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 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 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.

5.3 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 (Menazza & Murphy, 2016; Gliemann & Hellsten, 2019). Additionally, oestrogen can post-translationally modify proteins via G protein-coupled oestrogen receptor 1 (GPER) activation (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 (Figure 5-3).

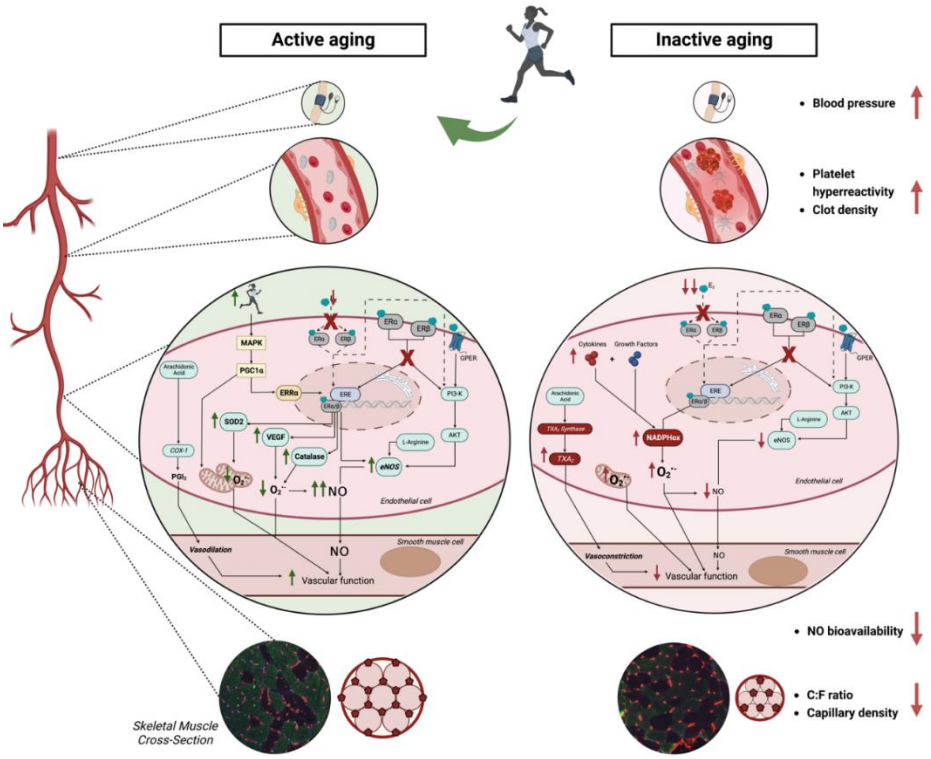

Figure 5-3: Outlined differences in vascular health with active aging versus inactive aging in women.

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 ($O^{-}2^{\bullet-}$), 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.

Interestingly, previous rodent (Novensà et al., 2011) and human (Novella *et al.*, 2012*b*) 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 *et al.*, 2012*b*). 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*b*; 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 (Perry *et al.*, 2014; Craige *et al.*, 2016). 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 and Hellsten (2019) (Figure 5-3).

5.4 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 (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 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 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.*, 2020*a*). 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.*, 2020*a*).

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 (Figure 5-4). 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 (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.

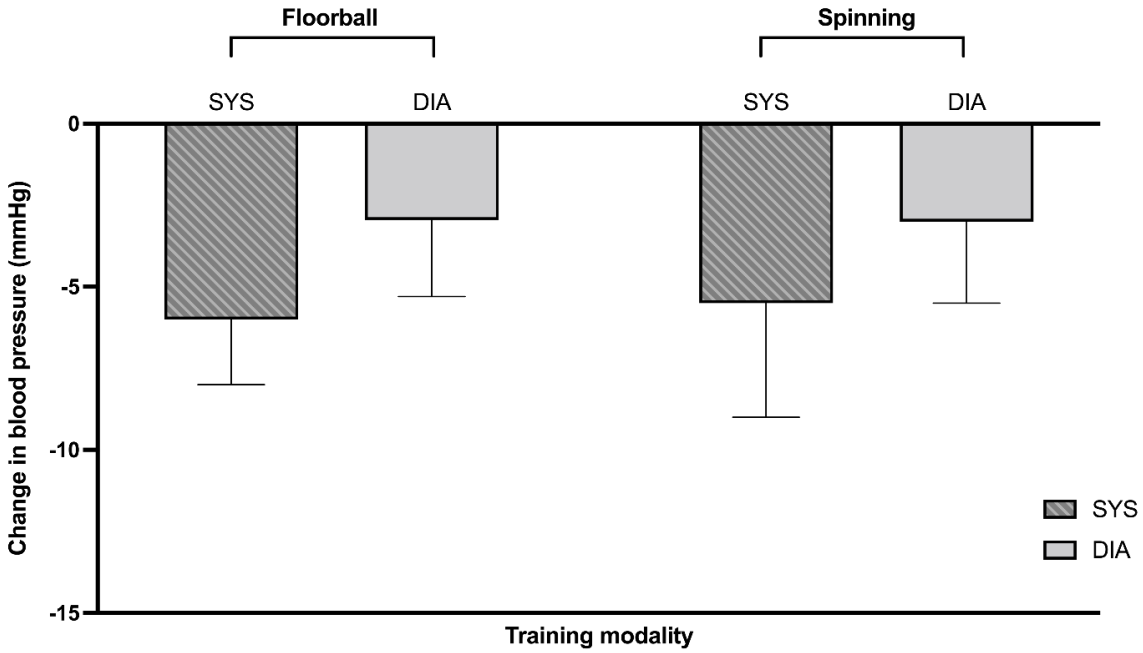

Figure 5-4: Reductions in clinical systolic and diastolic blood pressure by exercise intervention in sedentary postmenopausal women (floorball, n=18, Spinning, n=27).

Data adapted and combined from: Nyberg et al., 2014, 2016; Gunnarsson et al., 2020; Hoier et al., 2021.

5.5 Vascular Function

5.5.1 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 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.

5.5.2 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 (Nyberg *et al.*, 2016; Gliemann *et al.*, 2020*b*). 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 *et al.*, 2020*b*). 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.*, 2020*a*). 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 (2020*a*),

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.

5.6 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.

5.6.1 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 *et al.*, 2012*a*). 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 PGI_2 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 postmenopausal women. Notably, exercise training has been shown to increase circulating NO (Zaros *et al.*, 2009; Esmail *et al.*, 2011) and PGI_2 (Gliemann *et al.*, 2020*b*) 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.

5.6.2 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 (Figure 5-5). 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 ~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.

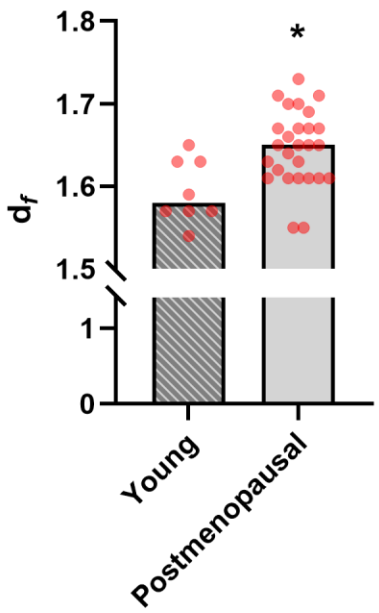

Figure 5-5: Fractal dimension in young and postmenopausal women.

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.

5.7 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 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; 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 $\dot{V}O_{2max}$) 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 lifelong 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 ($R^2 = 0.12$; $p = 0.007$) (Figure 5-6). Interestingly, this relationship does not apply to lifelong-trained women (Figure 5-6) (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.

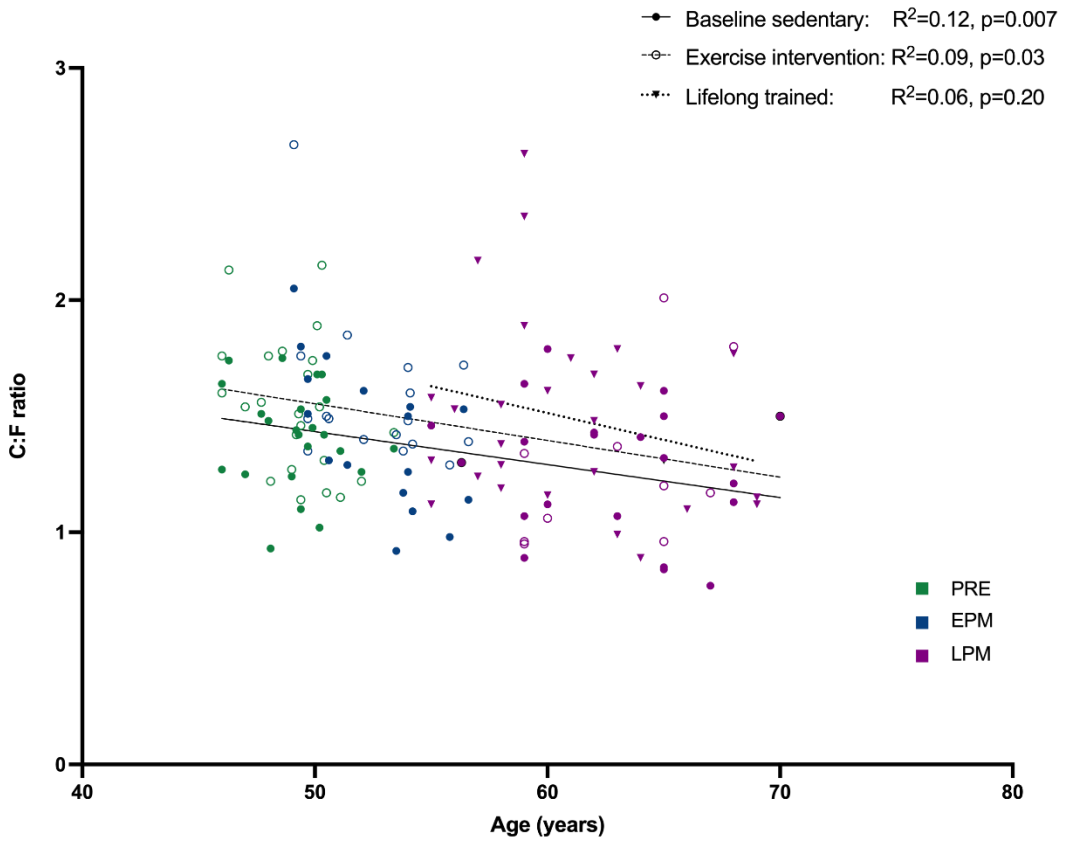

Figure 5-6: Relationship between age (years) and capillary-to-fiber (C:F) ratio.

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 of 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 pr week, highly: more than 4 hours of moderate- and high-intensity exercise pr. 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.

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 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.

5.8 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.

5.9 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 (Figure 5-3). 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.

5.10 References

Akerstrom T, Laub L, Vedel K, Brand CL, Pedersen BK, Lindqvist AK, Wojtaszewski JFP & Hellsten Y (2014). Increased skeletal muscle capillarization enhances insulin sensitivity. *Am J Physiol Endocrinol Metab*; DOI: 10.1152/ajpendo.00020.2014.

Barton M & Meyer MR (2009). Postmenopausal hypertension: Mechanisms and therapy. *Hypertension*; DOI: 10.1161/HYPERTENSIONAHA.108.120022.

le Blanc J & Lordkipanidzé M (2019). Platelet Function in Aging. *Front Cardiovasc Med*; DOI: 10.3389/fcvm.2019.00109.

di Blasio A, Ripari P, Bucci I, di Donato F, Izzicupo P, D'Angelo E, di Nenno B, Taglieri M & Napolitano G (2012). Walking training in postmenopause: Effects on both spontaneous physical activity and training-induced body adaptations. *Menopause*; DOI: 10.1097/gme.0b013e318223e6b3.

Bonner JS, Lantier L, Hasenour CM, James FD, Bracy DP & Wasserman DH (2013). Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance. *Diabetes* **62,** 572–580.

Bray PF (2007). Platelet Hyperreactivity: Predictive and Intrinsic Properties. *Hematol Oncol Clin North Am*; DOI: 10.1016/j.hoc.2007.06.002.

Bucciarelli P & Mannucci PM (2009). The hemostatic system through aging and menopause. *Climacteric*; DOI: 10.1080/13697130903006365.

Canonico M, Plu-Bureau G, O'Sullivan MJ, Stefanick ML, Cochrane B, Scarabin PY & Manson JE (2014). Age at menopause, reproductive history, and venous thromboembolism risk among postmenopausal women: The Women's Health Initiative hormone therapy clinical trials. *Menopause*; DOI: 10.1097/GME.0b013e31829752e0.

Cebula A, Tyka AK, Tyka A, Pałka T, Pilch W, Luty L & Mucha D (2020). Physiological response and cardiorespiratory adaptation after a 6-week Nordic Walking training targeted at lipid oxidation in a group of post-menopausal women. *PLoS One*; DOI: 10.1371/journal.pone.0230917.

Craige SM, Kröller-Schön S, Li C, Kant S, Cai S, Chen K, Contractor MM, Pei Y, Schulz E & Keaney JF (2016). PGC-1α dictates endothelial function through regulation of eNOS expression. *Sci Rep*; DOI: 10.1038/srep38210.

Desouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H & Seals DR (2000). *Regular Aerobic Exercise Prevents and Restores Age-Related Declines in*

Endothelium-Dependent Vasodilation in Healthy Men. Available at: http://www.circulationaha.org.

Egginton S (2009). Invited review: Activity-induced angiogenesis. *Pflugers Arch*; DOI: 10.1007/s00424-008-0563-9.

Esmail A, Mohamed U & Hamza F (2011). Effect of Treadmill Exercise on Nitric Oxide in Hypertensive Women. *Bulletin of Egyptian Society for Physiological Sciences*; DOI: 10.21608/besps.2011.36126.

Evans PA, Hawkins K, Lawrence M, Williams RL, Barrow MS, Thirumalai N & Williams PR (2008). Rheometry and associated techniques for blood coagulation studies. *Med Eng Phys*; DOI: 10.1016/j.medengphy.2007.08.005.

Faraco G & Iadecola C (2013). Hypertension: A harbinger of stroke and dementia. *Hypertension*; DOI: 10.1161/HYPERTENSIONAHA.113.01063.

Förstermann U & Münzel T (2006). Endothelial nitric oxide synthase in vascular disease: From marvel to menace. *Circulation*; DOI: 10.1161/CIRCULATIONAHA.105.602532.

Fredette NC, Meyer MR & Prossnitz ER (2018). Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. *Journal of Steroid Biochemistry and Molecular Biology*; DOI: 10.1016/j.jsbmb.2017.05.006.

Fuchs FD & Whelton PK (2020). High Blood Pressure and Cardiovascular Disease. *Hypertension*; DOI: 10.1161/HYPERTENSIONAHA.119.14240.

Gavin TP, Kraus RM, Carrithers JA, Garry JP & Hickner RC (2014). Aging and the Skeletal Muscle Angiogenic Response to Exercise in Women. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*; DOI: 10.1093/gerona/glu138.

Gliemann L & Hellsten Y (2019). The exercise timing hypothesis: can exercise training compensate for the reduction in blood vessel function after menopause if timed right? *Journal of Physiology* **597,** 4915–4925.

Gliemann L, Rytter N, Tamariz-Ellemann A, Egelund J, Brandt N, Carter HH & Hellsten Y (2020*a*). Lifelong Physical Activity Determines Vascular Function in Late Postmenopausal Women. *Med Sci Sports Exerc* **52,** 627–636.

Gliemann L, Rytter N, Yujia L, Tamariz-Ellemann A, Carter H & Hellsten Y (2021). A High Activity Level Is Required for Augmented Muscle Capillarization in Older Women. *Med Sci Sports Exerc* **53,** 894–903.

Gliemann L, Tamariz-Ellemann A, Baasch-Skytte T, Ehlers TS & Gunnarsson TP (2020*b*). Increased prostacyclin formation after high-intensity interval training in late postmenopausal women. *Eur J Appl Physiol* **120,** 1711–1720.

Green DJ, Jones H, Thijssen D, Cable NT & Atkinson G (2011). Flow-Mediated Dilation and Cardiovascular Event Prediction. *Hypertension*; DOI: 10.1161/hypertensionaha.110.167015.

Gries KJ, Raue U, Perkins RK, Lavin KM, Overstreet BS, D'Acquisto LJ, Graham B, Finch WH, Kaminsky LA, Trappe TA & Trappe S (2018). Cardiovascular and skeletal muscle health with lifelong exercise. *J Appl Physiol*; DOI: 10.1152/japplphysiol.00174.2018.

Gunnarsson TP, Ehlers TS, Baasch-Skytte T, Lund AP, Tamariz-Ellemann A, Gliemann L, Nyberg M & Bangsbo J (2020). Hypertension is associated with blunted NO-mediated leg vasodilator responsiveness that is reversed by high-intensity training in postmenopausal women. *Am J Physiol Regul Integr Comp Physiol*; DOI: 10.1152/AJPREGU.00170.2020.

Hearon CM & Dinenno FA (2016). Regulation of skeletal muscle blood flow during exercise in ageing humans. *Journal of Physiology* **594,** 2261–2273.

Hoier B, Nordsborg N, Andersen S, Jensen L, Nybo L, Bangsbo J & Hellsten Y (2012). Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. *Journal of Physiology*; DOI: 10.1113/jphysiol.2011.216135.

Hoier B, Olsen LN, Leinum M, Jørgensen TS, Carter HH, Hellsten Y & Bangsbo J (2021). Aerobic High-Intensity Exercise Training Improves Cardiovascular Health in Older Post-menopausal Women. *Frontiers in Aging*; DOI: 10.3389/fragi.2021.667519.

Holder SM, Brislane Á, Dawson EA, Hopkins ND, Hopman MTE, Cable NT, Jones H, Schreuder THA, Sprung VS, Naylor L, Maiorana A, Thompson A, Thijssen DHJ & Green DJ (2019). Relationship between endothelial function and the eliciting shear stress stimulus in women: Changes across the lifespan differ to men. *J Am Heart Assoc*; DOI: 10.1161/JAHA.118.010994.

Hyder SM, Huang JC, Nawaz Z, Boettger-Tong H, Mäkelä S, Chiappetta C & Stancel GM (2000). Regulation of vascular endothelial growth factor expression by estrogens and progestins. *Environ Health Perspect*; DOI: 10.2307/3454307.

Ighodaro OM & Akinloye OA (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*; DOI: 10.1016/j.ajme.2017.09.001.

Kaibara M (1996). Rheology of blood coagulation. *Biorheology*; DOI: 10.1016/0006-355X(96)00010-8.

Kim YW & Byzova T v. (2014). Oxidative stress in angiogenesis and vascular disease. *Blood*; DOI: 10.1182/blood-2013-09-512749.

Landers-Ramos RQ & Prior SJ (2018). The Microvasculature and Skeletal Muscle Health in Aging. *Exerc Sport Sci Rev*; DOI: 10.1249/JES.0000000000000151.

Lawrence MJ, Sabra A, Thomas P, Obaid DR, D'Silva LA, Morris RHK, Hawkins K, Brown MR, Williams PR, Davidson SJ, Chase AJ, Smith D & Evans PA (2015). Fractal dimension: A novel clot microstructure biomarker use in ST elevation myocardial infarction patients. *Atherosclerosis*; DOI: 10.1016/j.atherosclerosis.2015.04.012.

Lima R, Wofford M & Reckelhoff JF (2012). Hypertension in postmenopausal women. *Curr Hypertens Rep* **14,** 254–260.

Liu S, Ding T, Liu H & Jian L (2018). GPER was associated with hypertension in post-menopausal women. *Open Medicine (Poland)*; DOI: 10.1515/med-2018-0051.

Liu Y, Christensen PM, Hellsten Y & Gliemann L (2022). Published ahead of Print Effects of Exercise Training Intensity and Duration on Skeletal Muscle Capillarization in Healthy Subjects: A Meta-analysis. ; DOI: 10.1249/MSS.0000000000002955.

Lundberg Slingsby MH, Gliemann L, Thrane M, Rytter N, Egelund J, Chan M v., Armstrong PC, Warner TD & Hellsten Y (2018). Platelet responses to pharmacological and physiological interventions in middle-aged men with different habitual physical activity levels. *Acta Physiologica*; DOI: 10.1111/apha.13028.

Lundberg Slingsby MH, Nyberg M, Egelund J, Mandrup CM, Frikke-Schmidt R, Kirkby NS & Hellsten Y (2017). Aerobic exercise training lowers platelet reactivity and improves platelet sensitivity to prostacyclin in pre- and postmenopausal women. *Journal of Thrombosis and Haemostasis*; DOI: 10.1111/jth.13866.

Meilahn EN, Kuller LH, Matthews KA & Kiss JE (1992). Hemostatic factors according to menopausal status and use of hormone replacement therapy. *Ann Epidemiol*; DOI: 10.1016/1047-2797(92)90094-7.

Menazza S & Murphy E (2016). The expanding complexity of estrogen receptor signaling in the cardiovascular system. *Circ Res* **118,** 994–1007.

Mennuni S, Rubattu S, Pierelli G, Tocci G, Fofi C & Volpe M (2014). Hypertension and kidneys: Unraveling complex molecular mechanisms underlying hypertensive renal damage. *J Hum Hypertens*; DOI: 10.1038/jhh.2013.55.

Moreau KL & Hildreth KL (2014). Vascular Aging across the Menopause Transition in Healthy Women. *Adv Vasc Med* **2014,** 1–12.

Moreau KL, Hildreth KL, Meditz AL, Deane KD & Kohrt WM (2012). Endothelial function is impaired across the stages of the menopause transition in healthy women. *Journal of Clinical Endocrinology and Metabolism* **97,** 4692–4700.

Moreau KL, Stauffer BL, Kohrt WM & Seals DR (2013*a*). Essential role of estrogen for improvements in vascular endothelial function with endurance exercise in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **98,** 4507–4515.

Moreau KL, Stauffer BL, Kohrt WM & Seals DR (2013*b*). Essential role of estrogen for improvements in vascular endothelial function with endurance exercise in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* **98,** 4507–4515.

Novella S, Dantas AP, Segarra G, Medina P & Hermenegildo C (2012*a*). Vascular aging in women: Is estrogen the fountain of youth? *Front Physiol*; DOI: 10.3389/fphys.2012.00165.

Novella S, Heras M, Hermenegildo C & Dantas AP (2012*b*). Effects of estrogen on vascular inflammation: A matter of timing. *Arterioscler Thromb Vasc Biol*; DOI: 10.1161/ATVBAHA.112.250308.

Novensà L, Novella S, Medina P, Segarra G, Castillo N, Heras M, Hermenegildo C & Dantas AP (2011*a*). Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ERα/ERβ balance in female mice. *PLoS One*; DOI: 10.1371/journal.pone.0025335.

Novensà L, Novella S, Medina P, Segarra G, Castillo N, Heras M, Hermenegildo C & Dantas AP (2011*b*). Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ERα/ERβ balance in female mice. *PLoS One*; DOI: 10.1371/journal.pone.0025335.

Nyberg M, Egelund J, Mandrup CM, Andersen CB, Hansen KMBE, Hergel IMF, Valbak-Andersen N, Frikke-Schmidt R, Stallknecht B, Bangsbo J & Hellsten Y (2017). Leg vascular and skeletal muscle mitochondrial adaptations to aerobic high-intensity exercise training are enhanced in the early postmenopausal phase. *Journal of Physiology* **595,** 2969–2983.

Nyberg M, Egelund J, Mandrup CM, Nielsen MB, Mogensen AS, Stallknecht B, Bangsbo J & Hellsten Y (2016). Early Postmenopausal Phase is Associated with Reduced Prostacyclin-Induced Vasodilation That is Reversed by Exercise Training: The Copenhagen Women Study. *Hypertension* **68,** 1011–1020.

Nyberg M, Jensen LG, Thaning P, Hellsten Y & Mortensen SP (2012). Role of nitric oxide and prostanoids in the regulation of leg blood flow and blood pressure in humans with essential hypertension: Effect of high-intensity aerobic training. *Journal of Physiology* **590,** 1481–1494.

Nyberg M, Seidelin K, Rostgaard Andersen T, Neumann Overby N, Hellsten Y, Bangsbo J & Biomarkers BJ (2014). Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise training. *Am J Physiol Regul Integr Comp Physiol* **306,** 510–517.

Nystoriak MA & Bhatnagar A (2018). Cardiovascular Effects and Benefits of Exercise. *Front Cardiovasc Med*; DOI: 10.3389/fcvm.2018.00135.

Olsen LN, Hoier B, Hansen C v., Leinum M, Carter HH, Jorgensen TS, Bangsbo J & Hellsten Y (2020). Angiogenic potential is reduced in skeletal muscle of aged women. *Journal of Physiology* **598,** 5149–5164.

Park YM, Pereira RI, Erickson CB, Swibas TA, Kang C & van Pelt RE (2017). Time since menopause and skeletal muscle estrogen receptors, PGC-1α, and AMPK. *Menopause* **24,** 815–823.

Parker BA, Kalasky MJ & Proctor DN (2010). Evidence for sex differences in cardiovascular aging and adaptive responses to physical activity. *Eur J Appl Physiol* **110,** 235–246.

Perez-Gomez J, Rytter N, Mandrup C, Egelund J, Stallknecht B, Nyberg M & Hellsten Y (2021). Menopausal transition does not influence skeletal muscle capillary growth in response to cycle training in women. *J Appl Physiol* **131,** 369–375.

Periayah MH, Halim AS & Saad AZM (2017). Mechanism action of platelets and crucial blood coagulation pathways in Hemostasis. *Int J Hematol Oncol Stem Cell Res*.

Perry MC, Dufour CR, Tam IS, B'chir W & Giguère V (2014). Estrogen-related receptor-α coordinates transcriptional programs essential for exercise tolerance and muscle fitness. *Molecular Endocrinology*; DOI: 10.1210/me.2014-1281.

Pierce GL, Eskurza I, Walker AE, Fay TN & Seals DR (2011). Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults. *Clin Sci* **120,** 13–23.

Piróg MM, Milewicz T, Jach R & Undas A (2016). Plasma fibrin clot properties in postmenopausal women: Effects of hormone therapy. *Menopause*; DOI: 10.1097/GME.0000000000000585.

Prossnitz ER & Barton M (2011). The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol*; DOI: 10.1038/nrendo.2011.122.

Ribon-Demars A, Pialoux V, Boreau A, Marcouiller F, Larivière R, Bairam A & Joseph V (2019). Protective roles of estradiol against vascular oxidative stress in ovariectomized female rats exposed to normoxia or intermittent hypoxia. *Acta Physiologica*; DOI: 10.1111/apha.13159.

Roach REJ, Lijfering WM, Rosendaal FR, Cannegieter SC & le Cessie S (2014). Sex difference in risk of second but not of first venous thrombosis: Paradox explained. *Circulation*; DOI: 10.1161/CIRCULATIONAHA.113.004768.

Sabetta A, Lombardi L & Stefanini L (2022). Sex differences at the platelet–vascular interface. *Intern Emerg Med*; DOI: 10.1007/s11739-022-02994-y.

Sabra A, Lawrence MJ, Aubrey R, Obaid D, Chase A, Smith D, Thomas P, Storton S, Davies GR, Hawkins K, Williams PR, Morris K & Evans PA (2017). Characterisation of clot microstructure properties in stable coronary artery disease. *Open Heart*; DOI: 10.1136/openhrt-2016-000562.

Santos-Parker JR, Strahler TR, Vorwald VM, Pierce GL & Seals DR (2017). Habitual aerobic exercise does not protect against micro-or macrovascular endothelial dysfunction in healthy estrogen-deficient postmenopausal women. *J Appl Physiol*; DOI: 10.1152/japplphysiol.00732.2016.

Seals DR, Nagy EE & Moreau KL (2019). Aerobic exercise training and vascular function with ageing in healthy men and women. *Journal of Physiology* **597,** 4901–4914.

Singla A, Bliden KP, Jeong YH, Abadilla K, Antonino MJ, Muse WC, Mathew DP, Bailon O, Tantry US & Gurbel PA (2013). Platelet reactivity and thrombogenicity in postmenopausal women. *Menopause*; DOI: 10.1097/gme.0b013e31825ebafd.

Sisson SB & Katzmarzyk PT (2008). International prevalence of physical activity in youth and adults. *Obesity Reviews*; DOI: 10.1111/j.1467-789X.2008.00506.x.

Staessen JA, Ginocchio G, Thijs L & Fagard R (1997). Conventional and ambulatory blood pressure and menopause in a prospective population study. *J Hum Hypertens*; DOI: 10.1038/sj.jhh.1000476.

Stein RA, Gaillard S & McDonnell DP (2009). Estrogen-related receptor alpha induces the expression of vascular endothelial growth factor in breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology*; DOI: 10.1016/j.jsbmb.2009.02.010.

Taddei S, Virdis A, Ghiadoni L, Mattei P, Sudano I, Bernini G, Pinto S & Salvetti A (1996). Menopause is associated with endothelial dysfunction in women. *Hypertension* **28,** 576–582.

Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A & Salvetti A (2001). *Age-Related Reduction of NO Availability and Oxidative Stress in Humans*. Available at: http://www.hypertensionaha.org.

Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo CB, Sudano I & Salvetti A (1995). Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation*; DOI: 10.1161/01.CIR.91.7.1981.

Wendelboe AM & Raskob GE (2016). Global Burden of Thrombosis: Epidemiologic Aspects. *Circ Res*; DOI: 10.1161/CIRCRESAHA.115.306841.

Wiik A, Hellsten Y, Berthelson P, Lundholm L, Fischer H & Jansson E (2009). Activation of estrogen response elements is mediated both via estrogen and muscle contractions in rat skeletal muscle myotubes. *Am J Physiol Cell Physiol*; DOI: 10.1152/ajpcell.00148.2008.

Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense H-W, Joffres M, Kastarinen M, Poulter N, Primatesta P, Rodríguez-Artalejo F, Stegmayr B, Thamm M, Tuomilehto J, Vanuzzo D & Vescio F (n.d.). *Hypertension Prevalence and Blood Pressure Levels in 6 European Countries, Canada, and the United States*. Available at: https://jamanetwork.com/.

Zaros PR, Pires CEMR, Bacci M, Moraes C & Zanesco A (2009). Effect of 6-months of physical exercise on the nitrate/nitrite levels in hypertensive postmenopausal women. *BMC Womens Health*; DOI: 10.1186/1472-6874-9-17.

5.11 Research Program Progression

In *Chapter 5*, we synthesized and critically evaluated the existing literature on the vascular adaptations to exercise training in postmenopausal women. Although exercise training is generally beneficial for everyone, we observed a trend for greater improvements in vascular health with exercise training in recently (≤5 years) compared to late (≥10 years) postmenopausal women, which supports the *exercise timing hypothesis*.

Accordingly, in *Chapter 6*, we wanted to experimentally validate the *exercise timing hypothesis* by exploring the efficacy of 8 weeks of high-intensity exercise training in recently and late postmenopausal women on a wide variety of markers of vascular health.

CHAPTER 6

Exercise training induces thrombogenic benefits in recently but not late postmenopausal women

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6.1 Abstract

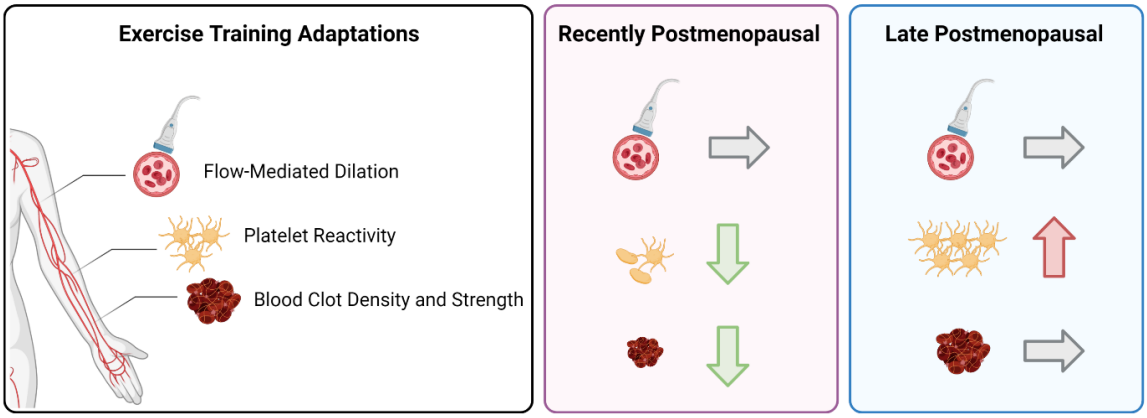

Figure 6-1: Study II graphical abstract.

Following 8 weeks of high-intensity exercise training, recently postmenopausal women obtain beneficial thrombogenic adaptations to the training period, whereas late postmenopausal women do not.

For women, both aging and menopause are significant risk factors for thrombotic events. Regular physical activity has been established as an effective long-term strategy to improve cardiovascular health in postmenopausal women. However, it is currently unclear whether the time passed after menopause prior to the initiation of exercise training impacts the magnitude of improvements in markers of thrombotic risk and conduit artery function in previously sedentary women. We hypothesized that the exercise-induced vascular improvements would be more pronounced in recently

compared to late postmenopausal women. We recruited 30 healthy postmenopausal women (15 recently and 15 late) and evaluated several markers of thrombotic risk and vascular health before and after an 8-week high-intensity exercise training intervention consisting of floorball and spinning performed 3 times per week. During the exercise training period, thrombotic risk, evaluated by platelet reactivity and fractal dimension, was reduced in the recently but not the late postmenopausal women. However, there was no change in conduit artery function, as measured by brachial and popliteal artery flow mediated dilation. The divergent thrombogenic adaptations may be partially explained by training-induced accrual of low-grade inflammation in the late postmenopausal women, as only this group exhibited increased intracellular adhesion molecule-1 levels after training. Taken together, these findings suggest that 8 weeks of high-intensity exercise training significantly reduces thrombotic risk in recently, but not late postmenopausal women. Thus, regular physical activity initiated soon after menopause, compared to many years after menopause, may be more efficient for reducing the risk of blood clots.

6.2 Introduction

Thrombotic events are the leading cause of death worldwide (Wendelboe and Raskob, 2016) and the risk of thrombosis is strongly linked to age, where the risk substantially increases after the age of 60 (Engbers et al., 2010). In women, the risk of thrombosis is further exacerbated by menopause and the concomitant cessation of estrogen production, which is known for its cardioprotective effects (Parker et al., 2010). It is well

established that estrogen has a robust capacity to mitigate oxidative stress via genomic and non-genomic estrogen receptor (ER) actions (Miller and Duckles, 2008). Together, these estrogenic effects can inhibit platelet activation and promote vasodilation, and may explain the lower incidence of cardiovascular disease in premenopausal women compared to age-matched men (Parker et al., 2010). Aging and menopause have been associated with a disproportional increase in the ratio of circulating factors that promote platelet activation compared to platelet inhibition (Le Blanc and Lordkipanidzé, 2019), an impaired capacity to degrade blood clots (Mari et al., 2008), and impaired vascular function (Seals et al., 2011). Notably, increased levels of oxidative stress as well as subsequent cellular damage and disturbance of function, are significant drivers of these menopause and aging-induced cardiovascular maladaptations (Xu et al., 2017). Due to the inevitable increase in the risk of cardiovascular events, both with age and menopause, it is critical to establish effective strategies to minimize this risk.

It is well-known that regular exercise is an effective strategy for eliciting robust beneficial effects on cardiovascular health (Gliemann et al., 2020; Nyberg et al., 2016; Nystoriak and Bhatnagar, 2018; Pedersen et al., 2018), and studies have documented the positive effects of exercise training on thrombotic risk in men (Schmidt et al., 2014; Vorup et al., 2017). However, data regarding how a brief period of exercise training influences the thrombogenic profile in postmenopausal women is limited and conflicting. Interestingly, when teased apart, it appears that studies showing a lack of improvements in vascular function with exercise training have typically included women that were a greater number of years postmenopausal (i.e., late postmenopausal) (Spina et al., 1993) or had a wide age

range of postmenopausal participants (Moreau et al., 2013; Pierce et al., 2011). Conversely, studies reporting substantial improvements in vascular function with exercise training have specifically recruited women who were recently postmenopausal (Egelund et al., 2017; Nyberg et al., 2016, 2014). The *exercise timing hypothesis* proposes that how soon exercise training is initiated after the onset of menopause may impact the magnitude of vascular adaptations (Gliemann and Hellsten, 2019). This notion is in line with evidence suggesting that hormone replacement therapy may only be effective at mitigating the development of cardiovascular disease if initiated soon after the onset of menopause (Miller et al., 2009).

Several potential mechanisms have been proposed to explain the *exercise timing hypothesis* in postmenopausal women (Gliemann and Hellsten, 2019). First, following the cessation of endogenous estrogen production with menopause, estrogen response elements (ERE) can still be activated by estrogen-related receptor alpha (ERRα) via contraction-mediated pathways, ultimately leading to changes in proteins important for vascular health (Miller and Duckles, 2008). However, the expression of ERRα may be reduced as a function of time after menopause (Francis and Pierce, 2011; Gliemann and Hellsten, 2019), suggesting that the capacity for upregulation of key vascular proteins may be lower in late postmenopausal women compared to recently postmenopausal women. Second, the magnitude of vascular impairments in late postmenopausal women may exceed those of recently postmenopausal women, making the vascular damage more difficult to reverse (Francis and Pierce, 2011). Hereunder, it is plausible that more severe vascular impairments are associated with increased susceptibility of oxidative stress

(Dhalla et al., 2000) and low-grade inflammation (Ross, 1999). Exercise acutely increases skeletal muscle reactive oxygen species production, an effect that is critical for exercise training adaptations (He et al., 2016). In healthy individuals, the endogenous antioxidant systems combat this acute elevation in reactive oxygen species, however with aging and menopause it is possible that reactive oxygen species formation exceeds the capacity of the antioxidant systems (Bejma and Ji, 1999), which may blunt the beneficial adaptations to exercise training.

To date, there is no experimental validation of the *exercise timing hypothesis* or the potential underlying mechanisms. Accordingly, the purpose of this study was to determine whether the timing of exercise training, as it relates to years after menopause, impacts the magnitude of improvements in markers of thrombotic risk and vascular function. We hypothesized that exercise-induced vascular improvements would be more pronounced in the recently compared to the late postmenopausal women, and that the underlying mechanism would be related to ERRα protein expression and/or inflammatory responses to exercise training.

6.3 Materials and Methods

The experimental protocol was approved by the ethics committee of Copenhagen (H-20037633) and was registered with ClinicalTrials.gov (NCT04596501). All experimental procedures were explained both verbally and in writing. Prior to participation, written informed consent was obtained and all experiments were conducted in accordance with the Declaration of Helsinki.

6.3.1 Recruitment of Participants

Participants were recruited via an online participant database and newspaper advertisements. Participants were included in the study if they were sedentary (no regular physical activity in >2 years and commuting <10 km daily by bicycle) but otherwise healthy postmenopausal women (>12 months since last menstrual bleeding, menopausal state was confirmed by blood hormone levels), 50 to 70 years old, body mass index (BMI) <30 $kg \cdot m^{-2}$, and normotensive (blood pressure <130/90 mmHg). The group of recently postmenopausal women were defined as >12 months since their last menstrual bleeding (i.e., menopausal) but ≤5 years postmenopausal. The group of late postmenopausal women were defined as ≥10 years postmenopausal. Accordingly, women that were >5 years but <10 years postmenopausal were excluded from this study. Additionally, participants were excluded if they smoked in the last 10 years, had excessive alcohol intake (>14 units per week), used regular medication, hormone replacement therapy (confirmed by blood hormone levels), or phytoestrogen supplementation (e.g., soybean or red clover products). Participants were recruited in two rounds with a balanced distribution of recently postmenopausal women and late postmenopausal women in each round. The first round started in the spring of 2021 and the second in the fall of 2021. This was done to accommodate for any potential seasonal/yearly variations in dietary and exercise habits.

6.3.2 Study Design

The study design consisted of a health screening day, a fitness assessment day, an experimental day, and 8 weeks of high-intensity exercise training. The fitness assessment

day and experimental day were performed before and after the training intervention with at least 48 h between these two visits. For all laboratory visits, participants were instructed to avoid caffeine for 24 h, strenuous exercise for 48 h, and non-steroidal anti-inflammatory drugs for at least 7 days prior to participation. Failure to comply with these guidelines resulted in rescheduling of the laboratory visit.

6.3.3 Health Screening Day

Prior to inclusion, the participants underwent a medical examination to determine their health status, eligibility to participate in exercise training, and to ensure that all inclusion criteria were met. The medical examination included a 10-point resting electrocardiogram (ECG), a medical interview and examination, as well as blood samples to ensure participants met the inclusion criteria. Participants also completed the short version of the International Physical Activity Questionnaire (IPAQ) to quantify their self-reported physical activity habits. During this visit, participants were familiarized with the incremental test that was performed on the fitness assessment day, described below.

6.3.4 Exercise Training

The participants completed 8 weeks of high-intensity exercise training, which involved 1 h of training performed 3 times per week. The participants performed a minimum of 20 sessions, and a maximum of 26 sessions over the 8-week period. The training included a combination of small-sided floorball matches (Pedersen et al., 2022, 2018) and interval-based high-intensity cycle training (spinning). This mixed modality, high-intensity training program was chosen to avoid overuse injuries and to maintain high levels of motivation. Participants were required to participate in at least one session of floorball and one

session of spinning per week with the option to select the third session based on preference. Each training session was monitored by members of the research team and encouragement was provided to promote high-intensity exercise. Heart rate (HR) was monitored continuously (POLAR TEAM Pro, Polar Electro Oy, Finland) throughout the exercise sessions to ensure that a high exercise intensity was maintained (average HR of the entire session >75% of maximal HR (HR_{max})). HR data was quantified using time spent in HR zones based on percent of HR_{max} assessed during the incremental test described below. Zone 1 was ≤60% HR_{max}, Zone 2 was 61 to 70% HR_{max}, Zone 3 was 71 to 80% HR_{max}, Zone 4 was 81 to 90% HR_{max}, Zone 5 was ≥91% HR_{max}.

6.3.5 Fitness Assessment Days

Before and after the exercise training intervention, participants completed a fitness assessment day. Before the first visit, the participants recorded their diet and arrived at the laboratory in a post-prandial state (≥2 h since last light meal). They were instructed to repeat their diet for the post-intervention fitness assessment day. The participants rested in a supine position for 15 min and then blood samples were drawn from the antecubital vein for the determination of circulating hormones (estrogen, progesterone, luteinizing hormone, follicle stimulating hormone, testosterone), coagulation factors, cholesterol, and hematological markers (Table 2). All blood samples were analyzed at a hospital (Rigshospitalet) in Copenhagen, Denmark within 2 h of collection.

Thereafter, participants performed an incremental test on a cycle ergometer for determination of peak oxygen uptake ($\dot{V}O_{2peak}$). The test consisted of a 5 min warm-up at 50 Watts (W) followed by 25 $W \cdot min^{-1}$ increases until exhaustion. Participants then rested

for 10 min before completing a $\dot{V}O_{2peak}$ verification by cycling at 110% W_{max} until exhaustion. $\dot{V}O_{2peak}$ was accepted if participants had an end-exercise respiratory exchange ratio that exceeded 1.0 and an end-exercise heart rate (HR) that exceeded 220 minus the age of the participant. $\dot{V}O_{2peak}$ was calculated as the highest consecutive 30 s average recorded during the step-test to exhaustion or the verification at 110% W_{max}.

6.3.6 Blood Pressure Monitoring at Home

For the 3 days prior to each experimental day, participants were instructed to measure blood pressure at home. They monitored blood pressure in the supine position, in the morning upon waking up and in the evening prior to going to sleep. The participants were carefully educated on how to perform accurate blood pressure measurements by members of the research team. Three measurements of blood pressure were obtained and recorded at each time point. If the final blood pressure measurement was the lowest of the three measurements, the participants were instructed to perform an additional measurement, for a maximum of six measurements. Both before and after the intervention period, the average systolic blood pressure (SBP) and average diastolic blood pressure (DBP) of all home measurements were calculated.

6.3.7 Experimental Day

Before and after the intervention period, the participants completed an experimental day, where the participants arrived at the laboratory in a fasted state and underwent several tests including dual energy x-ray absorptiometry (DXA) scans, blood sampling,

flow-mediated dilation (FMD), and a biopsy was collected from m. vastus lateralis. The experimental procedures are described below.

6.3.8 Body Composition

The participants rested for 15 min in a supine position before a DXA scan was performed to assess body composition. The scan was performed with the participant wearing as little clothing as they felt comfortable, and this was replicated for the post-training scan. Then, the participants were escorted to another research laboratory for the remainder of the experimental protocol.

6.3.9 Blood Sampling

The participants rested in a dimly lit, temperate controlled (22-23°C) room in a supine position for 30 min before blood samples were collected from the antecubital vein for the following experimental procedures:

6.3.9.1 Incipient Clot Microstructure – Fractal Dimension

The density and strength of a blood clot is a valuable predictor of thrombotic risk and can be evaluated using rheometry (Lawrence et al., 2014). This novel method uses uncoagulated whole blood to form *in-vitro* blood clots. Fractal dimension is a quantitative measure of the incipient clot microstructure (i.e., density and mechanical strength), and is determined at the point where blood transitions from a viscoelastic fluid to a viscoelastic solid (gel point; GP) (Lawrence et al., 2014). To perform this measurement, a 9 mL blood sample was drawn via a butterfly needle (BD Vacutainer Push Button Blood Collection Set, 367344, BD, USA) into a blood collection tube with no additive (455001,

Greiner Bio-One, Austria) and care was taken to ensure that the tourniquet was on for as short a time as possible to prevent shear stress-induced platelet activation (Magnette et al., 2016). Then, 7 mL was immediately loaded into a double-gap concentric cylinder measuring geometry of a controlled stress rheometer (AR-G2, TA Instruments, USA) at 37°C. The blood was loaded by the same investigator using a reverse pipetting technique to avoid bubbles. Immediately after sample loading, small amplitude oscillatory shear measurements were performed at test frequencies: 2, 0.93, 0.43, and 0.2 Hz with a peak stress amplitude of 0.03 Pa to promote blood clot formation. The method determines the GP of the incipient blood clot, which acts as a template for ongoing clot development (Curtis et al., 2013, 2011). A detailed description of the physics and mathematical calculations behind the fractal dimension method can be found elsewhere (Lawrence et al., 2014).

6.3.9.2 Platelet Reactivity

Platelets and platelet aggregation are a critical component of blood clot formation and subsequent thrombosis (Periayah et al., 2017). To perform this assay, 12.5 mL of blood were drawn into 3.2% sodium-citrate blood collection tubes (454332, Greiner Bio-One, Austria) and immediately centrifuged at 180 g for 10 minutes at 20°C to obtain platelet rich plasma (PRP). The PRP was pipetted into a 96 well round bottom plate (650101, Greiner Bio-One, Austria), and 2 mL of the remaining blood was placed in an Eppendorf tube and centrifuged at 15000 g for 2 minutes at 20°C to obtain platelet poor plasma (PPP). The PPP was pipetted into the same 96 well round bottom plate. Then, as per our previous experiments (Lundberg Slingsby et al., 2018, 2017), 40 µL of PRP or PPP was

added via multi-channel pipette to a 96-well half-area plate (675161, Greiner Bio-One, Austria) pre-coated with known concentrations of the platelet agonists: thrombin receptor activator peptide 6 (TRAP6) [0.11 – 40 µM; TRAP6, 4017752, Bachem, Switzerland] and collagen [0.0156 – 16 µg·mL^{-1}; 1130630, Takeda, Japan]. All agonists were run in triplicate. The 96-well half-area plate was covered with Parafilm and placed on an orbital plate shaker (Thermomixer C, Eppendorf, Germany) for 5 min at 37°C and 1200 rpm to allow for optimal aggregation to occur. The 96-well half-area plate was immediately analyzed using a plate reader (Emax, Molecular Devices, USA), where absorbance was measured at 595 nm and platelet aggregation (%) was calculated:

$$\boldsymbol{Platelet\ aggregation}\ (\%) = 1 - \left(\frac{sample - PPP}{PRP - PPP}\right) x\ 100\%$$

Platelet aggregation (%) was calculated for each concentration of agonist and the area under the curve (AUC) was calculated as the integral under the entire platelet reactivity curve for each agonist.

6.3.9.3 Hematological Assessment and Plasma Storage

The hematological assessment was performed using a hematology analyzer (Sysmex XN-2100, Sysmex, Japan). A 3 mL blood sample was collected in an ethylenediaminetetraacetic acid (EDTA)-coated tube (454246, Greiner Bio-One, Austria) for determination of erythrocytes, white blood cells, hematocrit, hemoglobin, and immune cells. Platelet count was determined from a 3.5 mL blood sample collected in a 3.2% sodium-citrate-coated tube (454332, Greiner Bio-One, Austria) (Dastjerdi et al., 2006; Schuff-Werner et al., 2013). Following hematological assessment, the EDTA and

sodium-citrate tubes were centrifuged at 2880 g at 5°C for 5 minutes. Plasma was collected at stored at -80°C for future analysis.

6.3.10 Flow-Mediated Dilation (FMD)

After the blood sampling, the participant remained supine, resting quietly in the dark for an additional 20 min before undergoing the assessment of flow-mediated dilation (FMD). For all participants, the left arm and leg were used to evaluate brachial and popliteal artery FMD, respectively. The order for brachial and popliteal artery FMD assessment was randomized between participants and repeated after the intervention period. Duplex-Doppler ultrasound (Vivid E9, GE Healthcare, USA) was used to measure artery diameter and blood velocity during the FMD assessment, where B-mode ultrasound was used to locate the best artery image on the arm and leg. A 9 MHz linear array transducer (GE Healthcare, USA), operating at 4/8 MHz and 4.2 MHz Doppler frequencies, was used to insonate the arteries and the angle-correction of ≤60º was respected.

For determination of brachial artery FMD, a rapid inflation/deflation pneumatic cuff system (E-20 Rapid Cuff Inflator, D.E. Hokanson, USA) was positioned around the left arm directly below the olecranon process and scanning of the brachial artery was performed 2-4 cm above the antecubital fossa. For determination of popliteal artery FMD, participants were placed in a prone position and the rapid inflation/deflation pneumatic cuff system (E-20 Rapid Cuff Inflator, D.E. Hokanson, USA) was placed around the lower left leg, at the midpoint place, and scanning of the popliteal artery was performed a minimum of 2 cm above the cuff position. For each artery, one min of baseline was recorded followed by rapid cuff inflation to 250 mmHg, which was maintained for 5 min

until rapid cuff deflation. The recordings for the reactive hyperemia started 10 s before cuff deflation and continued for an additional 3 minutes after cuff deflation. A 10 min break was provided between brachial and popliteal artery measurements.

The changes in the vessel diameter were analyzed offline using specialized edge-detection and all-tracking software (Vascular Research Tools 5, Medical Imaging Applications LLC, USA). Brachial and popliteal artery diameters were averaged into 3 s time bins, and the average of the 60 s baseline is reported. The percentage change for the FMD was calculated based on peak diameter after the cuff deflation period and baseline value. Shear rate was calculated using the following equation:

$$\boldsymbol{Shear\ rate}\ (s^{-1}) = 4 * \left(\frac{V_{mean}(cm \cdot s^{-1})}{diameter\ (cm)}\right)$$

6.3.11 Skeletal Muscle Biopsies

Biopsies were obtained from the m. vastus lateralis muscle using a Bergström needle with suction after local anesthesia (Lidocaine, 20 mg·mL^{-1}, Astra Zeneca, Denmark) of the skin and fascia, as described previously (Bergstrom, 1975). All muscle samples were immediately frozen in liquid nitrogen and stored at -80°C until future western blot analysis.

6.3.12 Western Blotting

Approximately 20 to 50 mg of skeletal muscle was used to determine protein expression of estrogen and estrogen-related receptors as well as microvascular enzymes and proteins via western blot analysis, as previously described (Hoier et al., 2012). Important

information specific to this study is described briefly. All samples were loaded in duplicates and samples from the same subject were always loaded on the same gel. All samples were loaded onto 4-15% Criterion TGX Precast Midi Protein Gels (5671085, BioRad, USA), except when measuring total oxidative phosphorylation (OX-PHOS) where samples were loaded onto 16.5% Criterion Tris-Tricine Gels (3450065, BioRad, USA). TGX stain-free gels were used as loading controls, where equal protein loading was confirmed by total protein determination from the stain-free image. Primary antibodies for estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), estrogen-related receptor alpha (ERRα; 96°C for 3 min), superoxide dismutase 2 (SOD2; 96°C for 3 min), endothelial nitric oxide synthase (eNOS; 96°C for 3 min), prostacyclin synthase (PGI_2S; 96°C for 3 min), G protein-coupled receptor 1 (GPER), and total oxidative phosphorylation (OX PHOS) were utilized. Figure 6-2 contains information regarding the antibodies, such as product number, dilution, and blocking agents. Membrane staining was visualized by incubation with a chemiluminescent HRP substrate: Luminata Forte (ECL; Merck Millipore, Darmstadt, Germany) or SuperSignal™ West Femto Maximum Sensitivity Substrate (FEMTO; Thermo Fisher Scientific, MA, USA) (Table 1). Protein content was expressed as the mean of the duplicates and was presented in arbitrary units related to human standards normalized to the average of all samples loaded on the same gel.

Primary Antibody	Function	Supplier	Catalog No.	Dilution of Primary Antibody	Blocking Agent	Secondary Antibody	Dilution of Secondary Antibody	Detection Agent
ERα	Receptor that binds estrogenic compounds.	Cell Signaling	8644	1:1000	5% Milk, TBST	Goat Anti-Rabbit IgG	1:5000	ECL
ERβ	Receptor that binds estrogenic compounds.	R&D Systems	MAB-7106	1:500	TBST	Goat Anti-Mouse IgG	1:5000	ECL
ERRα	Estrogen-related receptor that can activate estrogen Response Elements. Estrogenic compounds can't be bound to ERRs.	Abcam	76223	1:750	2% Milk, TBST	Goat Anti-Rabbit IgG	1:5000	ECL
eNOS	Enzyme that is protective of the cardiovascular system, through the production of NO.	Abcam	5589	1:1000	5% Fish, TBST	Goat Anti-Rabbit IgG	1:5000	FEMTO
GPER	Plasma membrane receptor that binds estrogenic compounds.	Abcam	39742	1:1000	5% Milk, TBST	Goat Anti-Rabbit IgG	1:5000	ECL
GPX1	Intracellular antioxidant enzyme that reduces hydrogen peroxide into water.	Abcam	22604	1:1000	5% Milk, TBST	Goat Anti-Rabbit IgG	1:5000	ECL
NOX2	A superoxide generating enzyme that forms reactive oxygen species.	Abcam	129068	1:3000	3% BSA, TBST	Goat Anti-Rabbit IgG	1:5000	ECL
OX PHOS	All five complexes involved in aerobic energy production in the mitochondria	Abcam	110411	1:1000	5% Milk, TBST	Goat Anti-Mouse IgG	1:5000	ECL
PGI_2S	Enzyme that produces prostacyclin, which is a vasodilatory compound and plays an important role in cardiovascular disease.	Abcam	23668	1:300	3% BSA, TBST	Goat Anti-Rabbit IgG	1:5000	ECL
SOD2	Transforms toxic superoxide anions, a product of the mitochondrial electron transport chain, to hydrogen peroxide.	Merck Millipore	06-984	1:5000	2% Milk, TBST	Goat Anti-Rabbit IgG	1:5000	ECL

Figure 6-2: Overview of antibodies used for western blotting.

ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; ERRα, estrogen related-receptor alpha; eNOS, endothelial nitric oxide synthase; G coupled-protein receptor, GPER; oxidative phosphorylation, GPX1; glutathione peroxidase 1, NOX2; NADPH oxidase 2, OXPHOS; PGI2S, prostacylin synthase; SOD2, superoxide dismutase; BSA, bovine serum albumin; TBST, Tris-buffered saline tween; ECL, Luminata Forte; FEMTO, SuperSignal™ West Femto Maximum Sensitivity Substrate.

6.3.13 Plasma Analysis

Plasma concentrations of plasminogen activator inhibitor-1 (PAI-1; ab269373, Abcam, United Kingdom) and 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$; 515211, Cayman Chemical, USA) were determined via enzyme-linked immunosorbent assays, performed according to the manufacturer's instructions using EDTA plasma. 6-keto prostaglandin F1α (6-keto PGF1α) is a breakdown product, and validated marker, of plasma prostacyclin levels (Mitchell, 1978).

Plasma markers of vascular inflammation (vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), serum amyloid alpha (SAA), and C-reactive protein (CRP)) were measured using an electrochemiluminescent assay kit and EDTA

plasma in accordance with the manufacturer's instructions (V-PLEX Vascular Injury Panel 2, K15198D Meso Scale Diagnostics, USA).

6.3.14 Statistical Analysis

The sample size in each group was estimated by performing a power calculation with an α level of 0.05 and a power level of 0.8 for a 6.25% change in fractal dimension based on previous report measuring fractal dimension after acute exercise (Davies et al., 2016; Lawrence et al., 2018) and drug interventions (Lawrence et al., 2018). We recruited 15 recently postmenopausal women and 15 late postmenopausal women however, the final data set includes 14 recently postmenopausal women (one dropout due to COVID-19) and 13 late postmenopausal women (two dropouts due to injury and inability to commit to the training intervention). Statistical analyses were performed using R-studio (Version 4.1.2, R Foundation for Statistical Computing, Austria). All statistical analyses were performed using linear mixed models with a Tukey post-hoc test. Independent factors of training (i.e., pre- and post-training; repeated variable) and group (i.e., recently and late postmenopausal women; non-repeated variable), subject ID was used as random variation. Unpaired t-tests were used for single comparisons of between group characteristics, such as participant characteristics (i.e., age, time after menopause, height), training duration, and endocrinology. Figures were created using GraphPad Prism (GraphPad Software, Version 9.3.1, USA). Normality of the data was confirmed using Q-Q plots. Statistical significance was accepted at $p \leq 0.05$. Trends are reported for $p < 1.0$. Data are reported as mean ± standard deviation (SD). The final number of replicates is indicated in each figure legend.

6.4 Results

6.4.1 Participant Characteristics

Twenty-seven healthy, but sedentary, postmenopausal women completed the 8-week high-intensity training intervention (*n*=14 recently postmenopausal women and *n*=13 late postmenopausal women). There were no significant differences in the measured characteristics between the groups, except for age and years after menopause. Participant characteristics are provided in Figure 6-3.

	Recently Postmenopausal Women		Late Postmenopausal Women	
	PRE	*POST*	*PRE*	*POST*
Participant characteristics				
Age (years)	56 ± 3	-	62 ± 5#	-
Time after menopause (years)	4 ± 1	-	13 ± 4#	-
Height (cm)	167 ± 4	-	166 ± 6	-
Body mass (kg)	69.6 ± 8.2	68.4 ± 8.0*	71.3 ± 9.0	71.4 ± 8.9
Body mass index ($kg \cdot m^{-2}$)	24.9 ± 2.3	24.5 ± 2.4*	25.9 ± 2.2	25.9 ± 2.3
Systolic blood pressure (mmHg)	113 ± 3	115 ± 3	117 ± 3	117 ± 3
Diastolic blood pressure (mmHg)	69 ± 2	70 ± 2	75 ± 2	72 ± 2(*)
$\dot{V}O_{2peak}$ ($mL \cdot min^{-1}$)	1950 ± 302	2024 ± 344(*)	1823 ± 209	1912 ± 264*
Body composition				
Lean body mass (kg)	41.1 ± 2.3	41.4 ± 2.3	40.5 ± 4.5	41.4 ± 4.5*
Fat (%)	37.0 ± 6.4	35.5 ± 6.5*	39.7 ± 3.7	38.4 ± 4.6*
Android fat (%)	41.3 ± 8.2	39.0 ± 9.4*	45.4 ± 6.5	43.4 ± 9.2(*)
Gynoid fat (%)	42.2 ± 6.3	40.3 ± 6.2*	44.2 ± 3.3	42.7 ± 3.5*
Visceral fat (g)	696 ± 460	638 ± 425(*)	944 ± 389	916 ± 392(#)
Cardiovascular risk factors				
Total cholesterol ($mmol \cdot L^{-1}$)	5.8 ± 0.6	5.7 ± 0.7	5.8 ± 0.8	5.8 ± 0.7
Low density lipoproteins ($mmol \cdot L^{-1}$)	3.5 ± 0.5	3.3 ± 0.5	3.5 ± 0.8	3.5 ± 0.7
High density lipoproteins ($mmol \cdot L^{-1}$)	1.9 ± 0.4	1.8 ± 0.3	1.9 ± 0.5	1.9 ± 0.3
Glycated hemoglobin ($mmol \cdot L^{-1}$)	6.0 ± 0.3	6.0 ± 0.4	6.2 ± 0.5(#)	6.1 ± 0.5
Endocrinology				
Estradiol ($nmol \cdot L^{-1}$)	0.09 ± 0.0	-	0.09 ± 0.0	-
Progesterone ($IU \cdot L^{-1}$)	0.6 ± 0.0	-	0.6 ± 0.0	-
Testosterone ($nmol \cdot L^{-1}$)	0.45 ± 0.11	-	0.53 ± 0.30	-
LH ($IU \cdot L^{-1}$)	43.5 ± 18.3	-	35.3 ± 11.8	-
FSH ($IU \cdot L^{-1}$)	81.9 ± 33.8	-	80.9 ± 23.7	-
Markers of coagulation				
D-Dimer ($FEU \cdot L^{-1}$)	0.31 ± 0.03	0.32 ± 0.08	0.30 ± 0.00	0.32 ± 0.04(*)
Fibrinogen ($\mu mol \cdot L^{-1}$)	8.21 ± 1.42	8.78 ± 2.71	8.63 ± 1.12	8.02 ± 0.81(*)
APTT (s)	28.80 ± 2.11	28.69 ± 4.37	28.33 ± 3.31	27.92 ± 2.18
Factor II+VIII+X (INR)	0.97 ± 0.05	1.03 ± 0.06*	0.97 ± 0.09	1.02 ± 0.09*
Factor II+VIII+X ($IU \cdot mL^{-1}$)	1.08 ± 0.17	1.63 ± 2.51	1.12 ± 0.22	1.01 ± 0.19

Figure 6-3: Characteristics of recently and late postmenopausal women.

Characteristics of recently postmenopausal women (RPW; n=14) and late postmenopausal women (LPW; n=13) assessed before and after an exercise training intervention including general characteristics, body composition, cardiovascular risk factors, sex hormone levels, markers of coagulation as well as inflammatory proteins. *, indicates significant difference between before and after the training intervention (p≤0.05). #, indicates significant difference between groups (p≤0.05).

6.4.2 Exercise Training Program

The participants in both groups completed an average of 23 ± 2 training sessions over the 8-week period. The recently postmenopausal women completed 17 ± 6 floorball sessions and 6 ± 6 spinning sessions, whereas the late postmenopausal women completed 15 ± 6 floorball sessions and 8 ± 5 spinning sessions. The average duration of each training session was 52 ± 1 min for the recently postmenopausal women and 53 ± 2 min for the late postmenopausal women. Average time spent in the different HR zones during the training sessions is depicted in Figure 6-4. There were no differences between the groups.

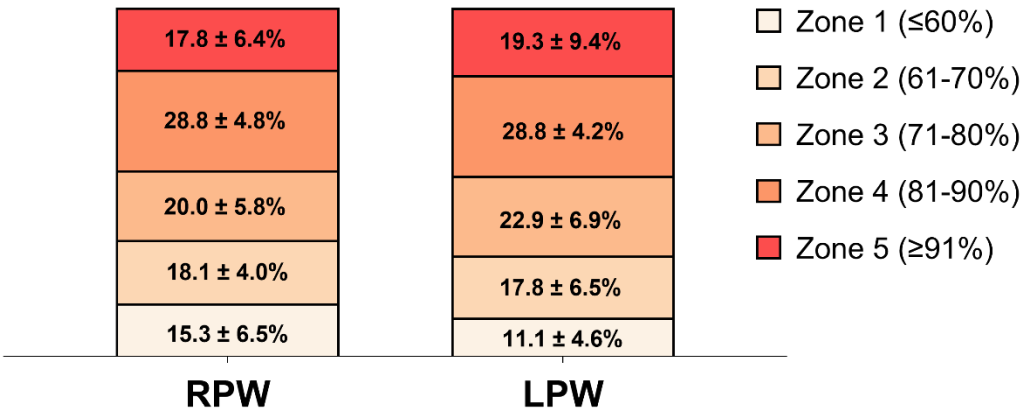

Figure 6-4: Average time spent in each training zone (%) over the 8-week high-intensity exercise training period (n=14 RPW and n=13 LPW).

The training zones were defined as, Zone 1: ≤60% HRmax, Zone 2: 61 to 70% HRmax, Zone 3: 71 to 80% HRmax, Zone 4: 81 to 90% HRmax, and Zone 5: ≥91% HRmax.

6.4.3 Peak Oxygen Uptake and Mitochondrial Proteins

Before training, there were no differences in $\dot{V}O_{2peak}$ and muscle OX-PHOS protein content. The late postmenopausal women improved $\dot{V}O_{2peak}$ during the training intervention period (by 5%; p=0.044), and increased muscle Complex I (by 54%; p=0.001), Complex II (by 56%; p=0.033), and Complex IV (by 63%; p=0.003) content. The recently

postmenopausal women did not present an increase in $\dot{V}O_{2peak}$ after, compared to before, the training intervention and muscle OX-PHOS expression remained unaltered in this group.

6.4.4 Platelet Reactivity

Before the training intervention, platelet reactivity to TRAP6 and collagen was not different between recently and late postmenopausal women. During the training intervention, platelet aggregation to [1.17 µM] TRAP6 significantly decreased in the recently postmenopausal women (by 31%; *p*=0.002; Figure 6-5A) and was unchanged in the late postmenopausal women. After the intervention, the late postmenopausal women had higher platelet aggregation to [1.17 µM] TRAP6 compared to recently postmenopausal women (by 33%; *p*=0.002). There was a main effect of group, where recently postmenopausal women had a lower TRAP6-induced platelet aggregation, measured as AUC, compared to late postmenopausal women (*p*=0.048). This was attributed to a trend for lower TRAP6-induced platelet aggregation in recently compared to late postmenopausal women after the training intervention (*p*=0.055). There was also a main effect of training, where TRAP6-induced platelet aggregation was lower after training (*p*=0.003). This was also driven by a reduction in aggregation in the recently postmenopausal women after compared to before training (by 11%; *p*=0.007; Figure 6-5E).

After the training intervention, platelet aggregation to [1.0 µg·mL^{-1}] collagen was increased in the late postmenopausal women (by 67%; *p*=0.001; Figure 6-5D). Moreover, after training, platelet aggregation in response to both [1.0 and 4.0 µg·mL^{-1}] collagen was

higher (p=0.001 and p=0.023, respectively), in late postmenopausal women compared to recently postmenopausal women. Similarly, after the training intervention, the late postmenopausal women demonstrated an increase in the AUC for collagen-induced aggregation compared to before training (p=0.025; Figure 6-5F).

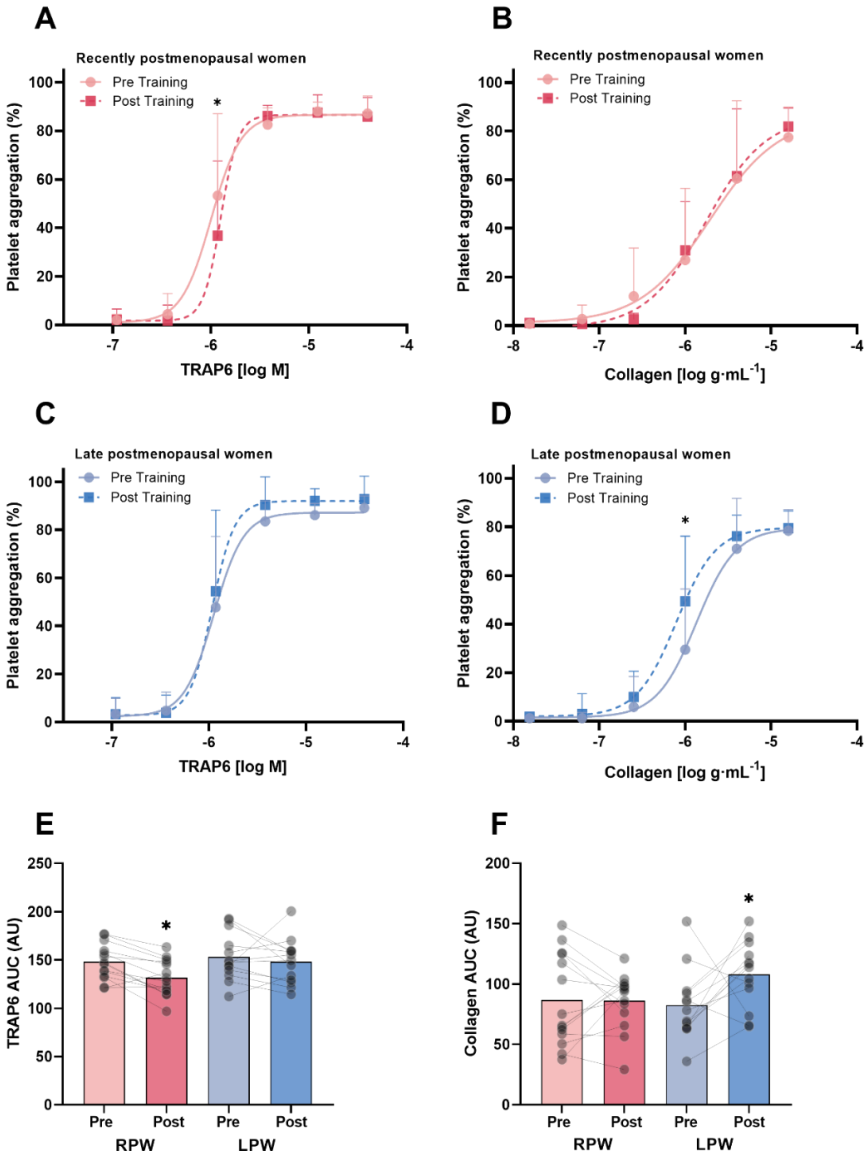

Figure 6-5: Platelet aggregation assays performed before and after training in recently postmenopausal women (RPW; n=14) and late postmenopausal women (LPW; n=13).

(**A**) Thrombin receptor activator peptide 6 (TRAP6)-induced platelet aggregation [0.11 to 40 µM] in RPW, (**B**) collagen-induced platelet aggregation [0.02 to 16 µg·mL-1] in RPW, (**C**) TRAP6-induced platelet aggregation in LPW, (**D**) collagen-induced platelet aggregation in LPW, (**E**) area under the curve (AUC) for TRAP6-induced platelet aggregation, and (**F**) AUC for collagen induced platelet aggregation. *, indicates significantly different compared to pre-training (p≤0.05).

6.4.5 Fractal Dimension

There was no difference in fractal dimension between recently and late postmenopausal women before the training intervention. There was a main effect of training, where fractal dimension was lower after, compared to before, the training intervention (p=0.018). This was driven by a decrease in fractal dimension in the recently postmenopausal women after, compared to before, the training intervention (1.61 ± 0.05 vs. 1.65 ± 0.05; p=0.027), whereas the late postmenopausal women showed no change (Figure 6-6).

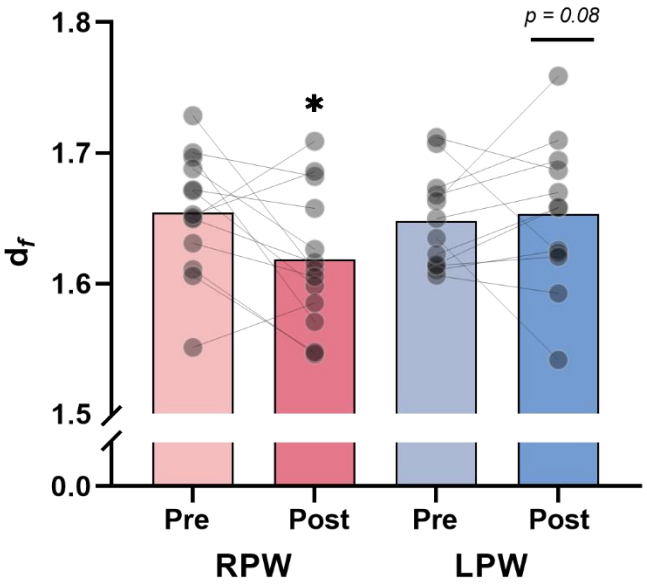

Figure 6-6: Fractal dimension (d_f) measured before and after the exercise training intervention in recently postmenopausal women (RPW; n=14) and late postmenopausal women (LPW; n=13).

*, indicates a significant reduction in fractal dimension after the training period (p≤0.05). Trend is presented comparing post-training fractal dimension between groups.

6.4.6 Markers of Coagulation

At baseline, there were no differences in markers of coagulation between the recently and late postmenopausal women (Figure 6-3). There was a main effect of training, where INR (a marker for clotting time) was increased post-training (p<0.001). Following the training intervention, both the recently (p=0.005) and the late (p=0.035) postmenopausal

women had increased international normalized ratio (INR), compared to before the intervention. No effect of the training intervention was observed for d-dimer, fibrinogen, activated partial thromboplastin clotting time (APTT) and coagulation factors (Figure 6-3).

6.4.7 Flow-Mediated Dilation

Before the training intervention, there was no difference between recently and late postmenopausal women for baseline diameter of the brachial (3.29 ± 0.30 vs. 3.36 ± 0.56 mm; Figure 6-7A) or popliteal (5.10 ± 0.77 vs. 5.02 ± 1.42 mm; Figure 6-7B) artery. Moreover, there were no baseline differences between recently and late postmenopausal women for brachial artery FMD (Figure 6-7C) or popliteal artery FMD (Figure 6-7D). Similarly, there were no differences between the recently and late postmenopausal women for brachial artery shear rate (173 ± 53 vs. 199 ± 67 s^{-1}) or popliteal artery shear rate (48 ± 32 vs. 49 ± 48 s^{-1}) prior to training.

There was a main effect of training, where baseline brachial artery diameter increased post-training (p<0.001). Specifically, in both the recently (p=0.002) and late (p=0.001) postmenopausal women, there was an increase in the baseline diameter of the brachial artery after training (Figure 6-7A). However, brachial artery FMD was unaffected by the training intervention (Figure 6-7C). Moreover, the training intervention did not change brachial artery shear rate in the recently (173 ± 53 vs. 197 ± 62 s^{-1}) or late postmenopausal women (199 ± 67 vs. 173 ± 47 s^{-1}).

After the training intervention, there was no change in the baseline diameter of the popliteal artery for the recently postmenopausal women, whereas the late postmenopausal women showed a trend for an increased popliteal artery baseline diameter (p=0.072; Figure 6-7B). The popliteal artery FMD did not change with the training intervention in the recently or the late postmenopausal women (Figure 6-7D). The training intervention did not alter popliteal artery shear rate in the recently (48 ± 32 vs. 60 ± 40 s^{-1}) or late (49 ± 48 vs. 35 ± 19 s^{-1}) postmenopausal women.

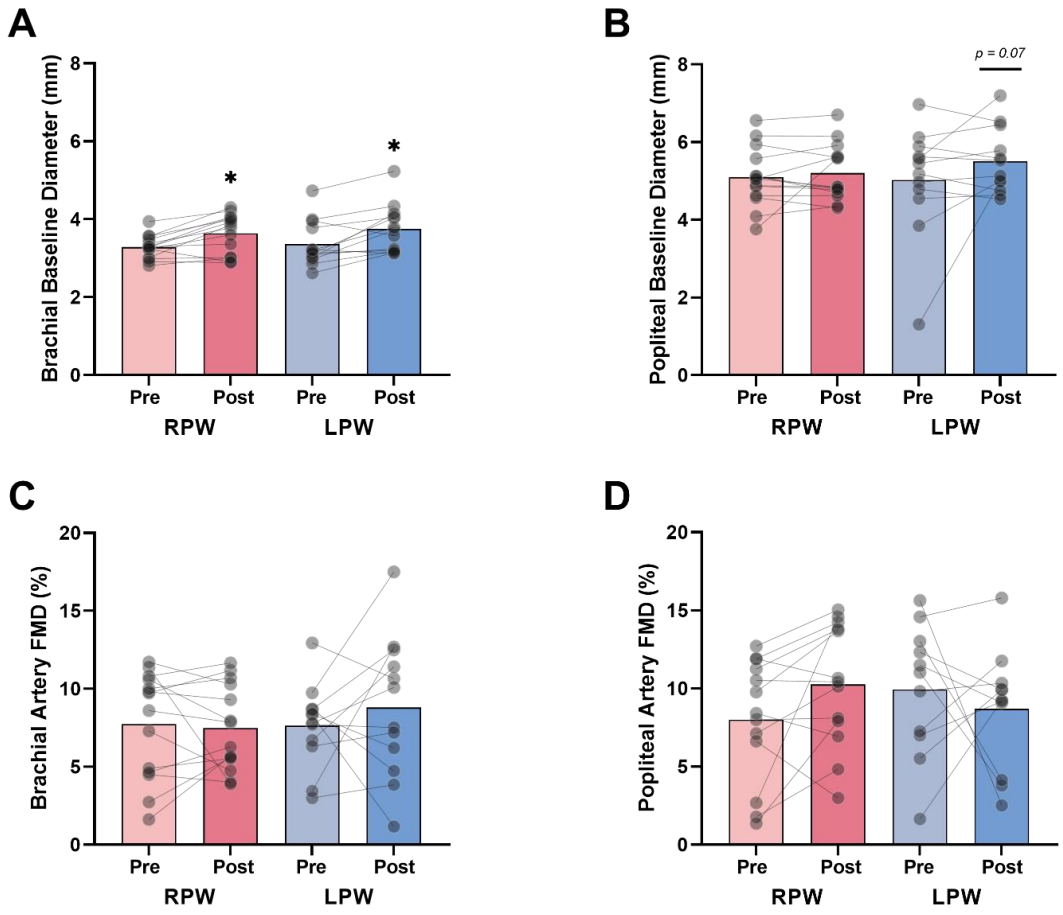

Figure 6-7: Flow-mediated dilation assessed in the brachial and popliteal arteries.

(**A**) Baseline diameter of the brachial artery and (**B**) baseline diameter of the popliteal artery. Flow-mediated dilation (FMD) expressed as a percentage change from baseline (%) measured at the (**C**) brachial artery and (**D**) popliteal artery in recently postmenopausal women (RPW; n=14) and late postmenopausal women (LPW; n=12) before and after exercise training. *, indicates a significant increase after training (p≤0.05). Trend is presented for training-induced increase within group.

6.4.8 Estrogen-Related Proteins

There were no differences in any pre-training estrogen-related protein expression, although there was a trend for higher muscle ERβ protein expression in the late compared to the recently postmenopausal women (p=0.057). There was a main effect of training, where ERα was higher after, compared to before, the training intervention (by 32%; p=0.017). This was mediated by increases in ERα protein expression in both the recently (p=0.025) and late postmenopausal women (p=0.007; Figure 6-8A). There was a main effect of the training intervention, where muscle ERβ was higher after, compared to before, the training intervention (by 24%; p<0.001). This was driven by an increase in ERβ protein expression in recently postmenopausal women (p=0.005), as there was no change in the late postmenopausal women (Figure 6-8B). After the training intervention there was a trend for an increase in the ratio of muscle ERα:ERβ compared to before the intervention in the late postmenopausal women (p=0.071), whereas the ratio was unaltered in the recently postmenopausal women. There was no change in muscle ERRα (Figure 6-8C) or GPER (Figure 6-8D) expression with the training intervention in either group. Representative blots are shown (Figure 6-8E).

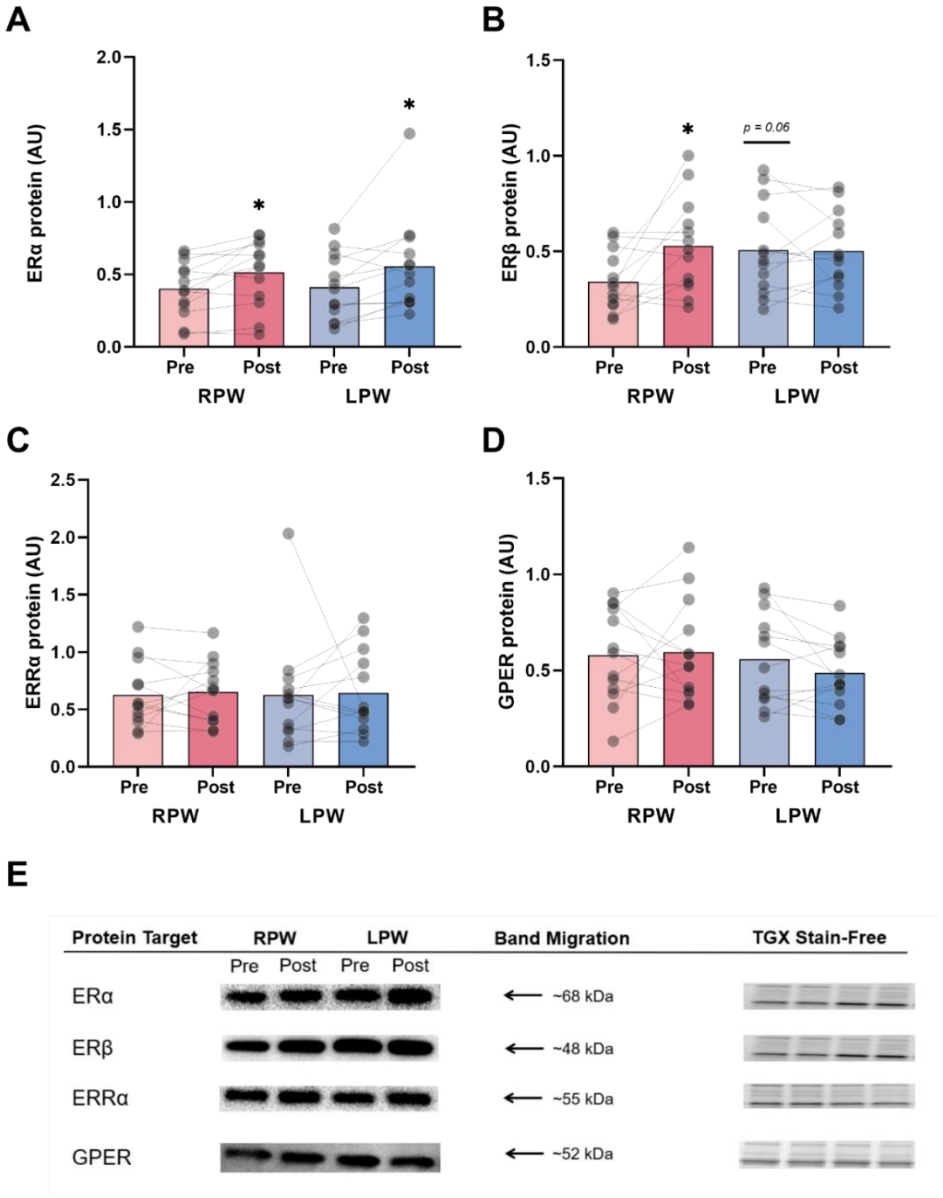

Figure 6-8: Protein expression of estrogen related proteins.

Protein expression of estrogen receptors, estrogen-related receptors, and g protein-coupled receptor from skeletal muscle biopsies obtained from the m. vastus lateralis of recently postmenopausal women (RPW; n=13) and late postmenopausal women (LPW; n=13) pre- and post-exercise training. (**A**) Estrogen receptor alpha (ERα), (**B**) estrogen receptor beta (ERβ), (**C**) estrogen-related receptor alpha (ERRα), (**D**) g protein-coupled receptor (GPER), and (**E**) representative blots and stain-free images. *, indicates significantly increased after training compared to before training (p≤0.05). Trend is presented comparing pre-training between groups.

6.4.9 Vascular Proteins

There were no differences in muscle vascular protein levels between the recently and late postmenopausal women at baseline (Figure 6-9). Muscle eNOS and PGI_2S protein expression did not change with the training intervention (Figure 6-9A, 6-9B). There was a main effect of the training intervention with muscle SOD2 protein content being higher after than before (by 52%; *p*=0.047). In the recently postmenopausal women, there was a trend for an increase in muscle SOD2 (*p*=0.068) and an increase in muscle SOD2 with the training intervention in the late postmenopausal women (*p*=0.001) (Figure 6-9C). There was no change in glutathione peroxidase 1 (GPX1; Figure 6-9D) or NADPH oxidase 2 (NOX2; Figure 6-9E) expression with the training intervention in either group. Representative blots are shown (Figure 6-9F).

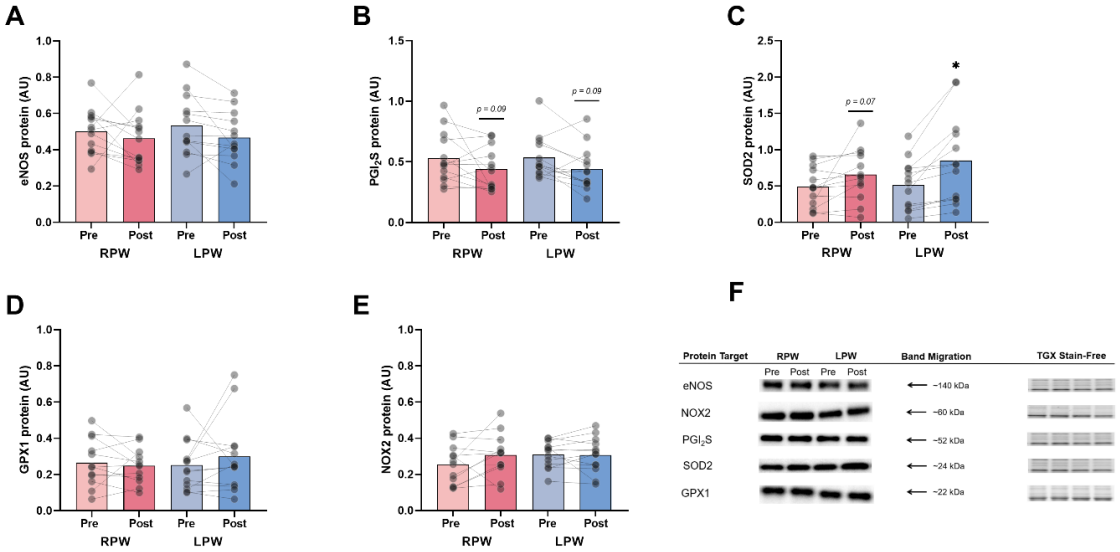

Figure 6-9: Protein expression of vascular proteins.

Protein expression of vascular proteins from skeletal muscle biopsies obtained from the m. vastus lateralis of recently postmenopausal women (RPW; n=13) and late postmenopausal women (LPW; n=13) before and after exercise training. (**A**) Endothelial nitric oxide synthase (eNOS), (**B**) prostacyclin synthase (PGI2S), (**C**) superoxide dismutase 2 (SOD2), (**D**) glutathione peroxidase 1 (GPX1), (**E**) NADPH oxidase 2 (NOX2), and (F) representative blots and stain-free images. *, indicates a significant increase compared to pre-training (p≤0.05). Trends are presented comparing pre- and post-training within group.

6.4.10 Plasminogen Activator Inhibitor-1 and 6-Keto Prostaglandin F1α (6-keto PGF1α) in Plasma

Before the training intervention, there was no difference in plasma PAI-1 levels between the recently and the late postmenopausal women (14.02 ± 15.70 vs. 17.33 ± 14.49 ng·mL^{-1}). The intervention did not alter plasma PAI-1 levels in the recently (11.59 ± 7.47 ng·mL^{-1}) or the late (15.69 ± 10.83 ng·mL^{-1}) postmenopausal women. Prior to the training intervention, there was no difference between the recently and late postmenopausal women for 6-keto $PGF_{1\alpha}$ (98.3 ± 93.7 vs. 67.8 ± 35.0 pg·mL^{-1}). Plasma 6-keto $PGF_{1\alpha}$ levels showed a main effect of the training intervention, where they were lower post-training (p=0.019). This was caused by a reduction in 6-keto $PGF_{1\alpha}$ in the recently postmenopausal women after the training intervention (70.2 ± 62.7 vs. 98.3 ± 93.7 pg·mL^{-1}; p=0.027), whereas there was no effect of training on plasma 6-keto $PGF_{1\alpha}$ in the late postmenopausal women (74.5 ± 44.6 vs. 67.8 ± 35.0 pg·mL^{-1}).

6.4.11 Plasma Markers of Vascular Health and Inflammation

ICAM-1 was not different between recently and late postmenopausal women. There was a main effect of training for increased ICAM-1 levels (p=0.019). This was driven by an increase in ICAM-1 after the training intervention in the late postmenopausal women only (p=0.022; Figure 6-10A). Prior to the training intervention, VCAM-1 was higher in the recently postmenopausal women compared to the late postmenopausal women (p=0.026; Figure 6-10B). There was a trend for VCAM-1 to increase in the late postmenopausal women after the training intervention (p=0.092), whereas there was no effect of the training intervention on VCAM-1 levels in the recently postmenopausal

women (Figure 6-10B). There was no baseline difference in CRP between the recently and late postmenopausal women and there was no effect of the training intervention (Figure 6-10C). No differences were observed in SAA levels between recently and late postmenopausal women or with the training intervention (Figure 6-10D).

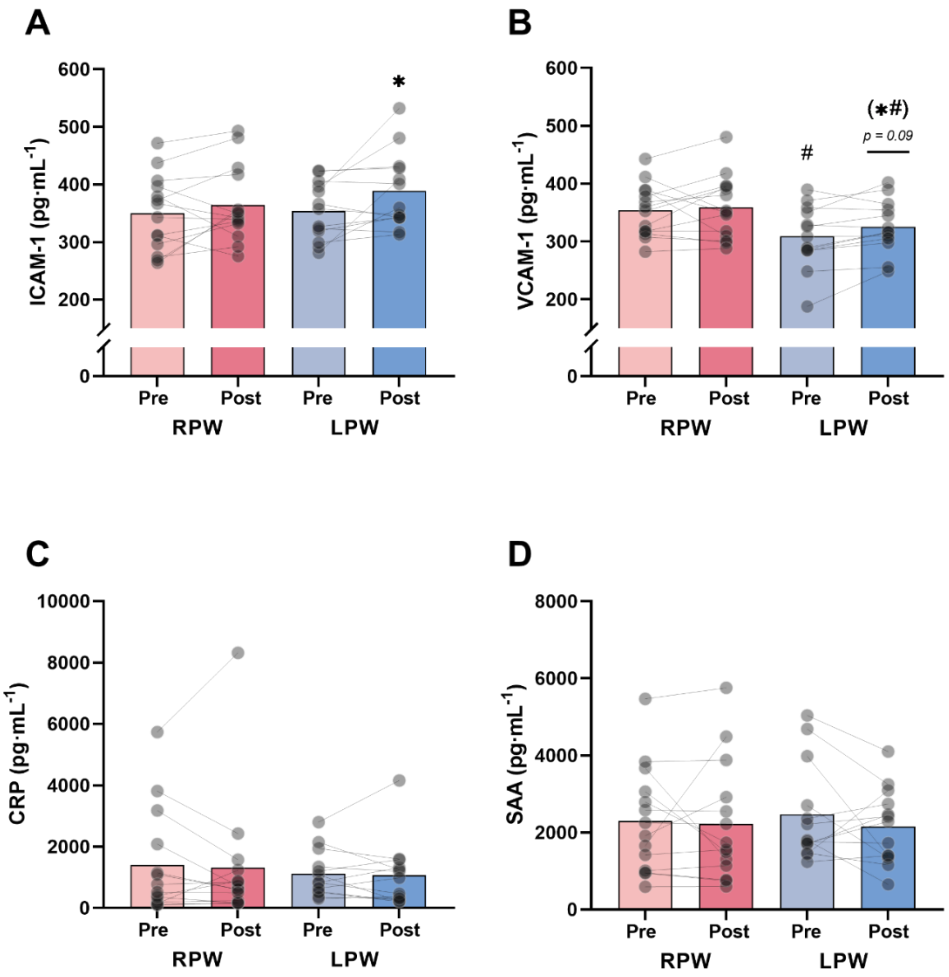

Figure 6-10: Plasma markers of vascular inflammation in recently postmenopausal (RPW; n=14) and late postmenopausal (n=13) women before and after exercise training.

Plasma markers of vascular inflammation in recently postmenopausal (RPW; n=14) and late postmenopausal (n=13) women before and after exercise training. (**A**) intracellular adhesion molecule-1 (ICAM-1), (**B**) vascular cell adhesion molecule-1 (VCAM-1), (**C**) C-reactive protein (CRP), and (**D**) serum amyloid alpha (SAA). *, indicates a significant increase compared to pre-training ($p \leq 0.05$). #, indicates a significant difference between recently and late postmenopausal women ($p \leq 0.05$). (*), indicates a trend for an increase compared to pre-training. (#), indicates a trend for a difference between groups after training.

6.4.12 Hematological Variables

There were no differences for baseline hematological markers between the recently and late postmenopausal women (Figure 6-11). After the exercise training intervention, hematocrit (p=0.019) and hemoglobin levels (p=0.027) were higher in the late postmenopausal women compared to the recently postmenopausal women.

Hematological assessments				
	RPW		**LPW**	
	PRE	*POST*	*PRE*	*POST*
Platelets ($\times10^3 \cdot \mu L^{-1}$)	185.6 ± 53.2	193.0 ± 40.1	184.4 ± 40.1	179.1 ± 41.1
Erythrocytes ($\times10^6 \cdot \mu L^{-1}$)	4.32 ± 0.30	4.24 ± 0.38	4.26 ± 0.25	4.30 ± 0.24
Hematocrit (%)	38.3 ± 2.4	37.4 ± 2.3	38.9 ± 1.7	39.4 ± 2.3#
Hemoglobin ($g \cdot dL^{-1}$)	13.0 ± 0.9	12.7 ± 0.7	13.2 ± 0.6	13.4 ± 0.7#
Leukocytes ($\times10^3 \cdot \mu L^{-1}$)	4.74 ± 0.80	4.86 ± 1.61	5.14 ± 1.03	5.19 ± 1.36
Lymphocytes ($\times10^3 \cdot \mu L^{-1}$)	1.62 ± 0.49	1.50 ± 0.38(*)	1.47 ± 0.36	1.48 ± 0.41
Monocytes ($\times10^3 \cdot \mu L^{-1}$)	0.36 ± 0.08	0.37 ± 0.10	0.44 ± 0.14(#)	0.47 ± 0.15(#)
Neutrophils ($\times10^3 \cdot \mu L^{-1}$)	2.60 ± 0.70	2.87 ± 1.31	3.02 ± 0.76	3.02 ± 1.03
Basophils ($\times10^3 \cdot \mu L^{-1}$)	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.05 ± 0.06(#)
Eosinophils ($\times10^3 \cdot \mu L^{-1}$)	0.12 ± 0.06	0.09 ± 0.05	0.17 ± 0.16	0.28 ± 0.25

Figure 6-11: Hematological variables in recently and late postmenopausal women.

Hematological variables in recently postmenopausal women (RPW; n=14) and late postmenopausal women (LPW; n=14 pre-training, n=13 post-training) measured before and after the exercise training period. *, indicates significant difference between before and after the training intervention (p≤0.05). #, indicates significant difference between groups (p≤0.05).

6.4.13 Markers of General Health

Body mass, body mass index, and resting blood pressure were similar between the two groups before the training intervention. There were main effects of the training intervention on body weight (p=0.012) and BMI (p=0.009), which were driven by significant reductions in body weight (p=0.018) as well as BMI (p=0.015) in the recently postmenopausal women, as there was no change in the late postmenopausal women

after the training intervention. The intervention did not change resting systolic or diastolic blood pressure in the recently or late postmenopausal women. In addition, the exercise training intervention did not alter any cardiovascular or metabolic risk factors including total cholesterol, low density lipoproteins, high density lipoproteins, or glycated hemoglobin in recently or late postmenopausal women (Figure 6-3).

6.4.14 Body Composition

Prior to the training intervention, there were no differences in body composition between the recently and late postmenopausal women. Lean body mass was significantly increased after the training intervention in the late postmenopausal women (p=0.002). In both groups, fat percentage was reduced with the training intervention (p=0.002). Both the recently postmenopausal women (p=0.005) and late postmenopausal women (p=0.016) had lower fat percentage after the training intervention compared to before training. Accordingly, there was a main effect of the training intervention to decrease android fat (p=0.022) and gynoid fat percentage (p<0.001). Specifically, the recently postmenopausal women demonstrated reductions in android fat (p=0.031) and gynoid fat percentage (p<0.001) as well as a trend for reduced visceral fat (p=0.089) after the training intervention. The late postmenopausal women showed a trend for reduced android fat percentage (p = 0.075), significantly reduced gynoid fat percentage (p=0.001), and no change in visceral fat. There was a trend for late postmenopausal women to have greater visceral fat compared to recently postmenopausal women after the training intervention (p=0.089) (Figure 6-3).

6.5 Discussion

The present study tested the hypothesis that the magnitude of thrombogenic and vascular adaptations to exercise training are blunted in women several years after menopause compared to soon after menopause. The main finding was that a period of high-intensity exercise training improved the thrombotic risk profile in recently postmenopausal women, as evidenced by reduced platelet reactivity and a less dense incipient clot microstructure. Conversely, these thrombogenic adaptations were not present in the late postmenopausal women, despite training-induced improvements in $\dot{V}O_{2peak}$ and OX-PHOS protein content. The lack of thrombogenic adaptations with training in the late postmenopausal women was paralleled by an increase in the level of the inflammatory plasma marker ICAM-1.

6.5.1 Late postmenopausal women are resistant to thrombogenic adaptations to exercise training

Although studies have investigated the effects of exercise training on vascular and thrombogenic adaptations in postmenopausal women (Hoier et al., 2021; Lundberg Slingsby et al., 2017; Moreau et al., 2013; Nyberg et al., 2016, 2014; Pierce et al., 2011), no studies have directly determined whether the magnitude of adaptations are impaired with increasing years after menopause. In agreement with our hypothesis, we observed a significant reduction in platelet aggregation to TRAP6 and a reduction in clot microstructure (i.e., lower fractal dimension) in recently, but not late postmenopausal women. This is the first study to show a training-induced reduction in platelet reactivity in recently postmenopausal women and the first to demonstrate a reduction in TRAP6-

induced platelet aggregation following exercise training. However, other investigations have shown lower collagen- and epinephrine-induced platelet aggregation in late premenopausal women (Lundberg Slingsby et al., 2017) and middle-aged men (Lundberg Slingsby et al., 2018) after exercise training. In addition, we assessed incipient clot microstructure via rheometry, which is a novel method to assess global thrombogenicity (Lawrence et al., 2014). For the first time, we show a training-induced reduction in fractal dimension, and thus clot microstructure of recently, but not late, postmenopausal women, suggesting that less dense blood clots are formed in the recently postmenopausal women. Critically, small changes in fractal dimension have been shown to reflect large changes in normalized blood clot mass (Sabra et al., 2017), where a reduction of 0.04, as observed in the recently postmenopausal women in this study, represents a ~70% drop in clot mass after training. Platelet activation and aggregation are important components of blood clot formation (Periayah et al., 2017) and accordingly, the observed reduction in platelet reactivity in the recently postmenopausal women may be a key contributor to the reduction in fractal dimension following exercise training. Moreover, it is possible that the increased platelet aggregation to collagen observed in the late postmenopausal women inhibited the exercise training-induced adaptations in fractal dimension. Together, the platelet reactivity and fractal dimension findings suggest that intense exercise training can reduce the propensity for thrombogenesis in recently postmenopausal women. In contrast, as these findings were absent in the late postmenopausal women, this population may be somewhat resistant to exercise-induced thrombogenic adaptations.

6.5.2 Improved Thrombogenic Risk Profile Without a Concomitant Improvement in Conduit Artery Function in Recently Postmenopausal Women

Previous studies have suggested that beneficial cardiovascular adaptations to exercise training are not as readily attained by postmenopausal women as by age-matched men (Moreau et al., 2013; Moreau and Hildreth, 2014; Santos-Parker et al., 2017). However, although not observed in the late postmenopausal women, the current training program successfully induced favourable thrombogenic adaptations in the recently postmenopausal women. Since the formation of NO and prostacyclin by the endothelium plays an important role in reducing platelet reactivity (Mitchell et al., 2008), we anticipated that an improved thrombogenic profile would reflect increased NO and prostacyclin bioavailability, and could translate to an improvement in endothelial function (Mitchell et al., 2008). However, this notion was not supported by the assessments of brachial artery and popliteal FMD, as there were no changes in either conduit artery with training. This finding could indicate that FMD is not a sufficiently sensitive measure for overall vascular formation of NO and prostacyclin, as these platelet inhibitors would be primarily produced in the microcirculation. Notably, although several previous investigations in postmenopausal women have demonstrated a lack of effect of training on conduit artery function (Hoier et al., 2021; Moreau et al., 2013; Pierce et al., 2011), a few studies have shown prostacyclin-mediated improvements in skeletal muscle microcirculatory function in postmenopausal women after a period of training (Nyberg et al., 2016) and with lifelong training (Gliemann et al., 2020). Thus, assessments of

microcirculatory endothelial function may be more relevant in the assessment of thrombogenic profile.

6.5.3 Potential Mechanisms Underpinning the Divergent Thrombogenic Responses to Exercise Training in Recently and Late Postmenopausal Women

We explored a few probable mechanisms that may underpin the divergent thrombotic responses to exercise training between the two groups including: (1) ER and/or ERRα signalling and (2) training-induced low-grade inflammation.

6.5.3.1 ER- and ERRα-signalling

ER and ERRα play critical roles in mitigating the accumulation of reactive oxygen species, inflammation and vascular damage (Puca et al., 2013), largely via the regulation of proteins (Ciana et al., 2003; Miller and Duckles, 2008; Wiik et al., 2009). Previous studies have highlighted that a decrease in ERα:ERβ favours a pro-inflammatory state (Novensà et al., 2011; Park et al., 2017), which may blunt vascular adaptations to exercise training. Although not statistically significant, we observed an increase in ERα protein expression with no change in ERβ in the late postmenopausal women and a consequent trend for an enhancement of the ratio of ERα:ERβ. Conversely, the ratio was unaltered by training in the recently postmenopausal women. Although speculative, it is possible that the ratio of ERα:ERβ is critical for the preservation/promotion of vascular health in menopause. Therefore, an increased ratio of ERα:ERβ may allow for faster exercise adaptation in

postmenopausal women and could explain the lack of thrombogenic adaptations in late postmenopausal women.

Contraction-mediated activation of ERRα may be critical for vascular health in postmenopausal women, as it has been shown to regulate some of the same pathways as endogenous estrogen, including key proteins related to vascular health and thrombogenesis (Ciana et al., 2003; Wiik et al., 2009). Interestingly, previous evidence suggests that ERRα content decreases with increasing years after menopause (Gliemann and Hellsten, 2019) and a period of exercise training can increase ERRα content in recently postmenopausal women (Nyberg et al., 2017). Surprisingly, we did not observe any differences in ERRα protein content between recently and late postmenopausal women, suggesting that the capacity for ERRα-contraction-mediated regulation was not different at baseline or altered by the training intervention. The present findings therefore do not support a critical role for ERRα protein content for the divergent thrombogenic adaptations to 8 weeks of high-intensity exercise training between recently and late postmenopausal women.

6.5.3.2 Vascular Inflammation

Previous evidence highlights that menopause-associated alterations of the vasculature, and in particular endothelial function, becomes progressively difficult to reverse with increasing time after menopause (Francis and Pierce, 2011; Hodis et al., 2016). This is often attributed to a progression from functional to structural alterations driven by chronic vascular inflammation (Francis and Pierce, 2011). After training, the late postmenopausal women had elevated levels of the inflammatory marker ICAM-1.

Although speculative, it is plausible that the exercise training period may have contributed to increased vascular stress and low-grade inflammation in the late postmenopausal women. Ultimately, this could have blunted the beneficial adaptations to the training program, a phenomenon that has been observed previously with overtraining (Smith, 2000). Notably, the elevated ICAM-1 levels in the late postmenopausal women after training may underpin the increased collagen-induced platelet reactivity after training, as inflammation can augment platelet reactivity (Stokes and Granger, 2012). However, a greater depth of research is required to definitively explain the mechanisms driving the divergent thrombogenic adaptations to exercise training in recently and late postmenopausal women.

6.5.4 Study Limitations

A limitation of the current study is that the exercise training intervention lasted only 8 weeks. It is unknown whether a longer period of training is required in late postmenopausal women to achieve the same magnitude of thrombogenic adaptations to exercise training as recently postmenopausal women, and future investigations are required to examine this issue. A second limitation is that, although the difference in the age of the recently (56 ± 3) and late postmenopausal (62 ± 5 years) women was minimal, the study design did not allow for separation between the influence of number of years after menopause and chronological age.

6.5.5 Conclusion

Our findings show that eight weeks of intense exercise training significantly improves the thrombotic risk profile in recently postmenopausal women, whereas these adaptations

may not be as readily achieved in late postmenopausal women. These findings indicate that initiating regular physical activity soon after menopause, compared to many years after menopause, may be more efficient for reducing the risk of blood clots. However, it remains to be elucidated whether late postmenopausal women simply require a longer period of exercise training to induce the same benefits as in recently postmenopausal women.

6.6 References

Adair, J.G., 1984. The Hawthorne effect: A reconsideration of the methodological artifact. Journal of Applied Psychology 69, 334–345. https://doi.org/10.1037/0021-9010.69.2.334

Alfarisi, H.A.H., Mohamed, Z.B.H., Ibrahim, M.B., 2020. Basic pathogenic mechanisms of atherosclerosis. Egyptian Journal of Basic and Applied Sciences 7, 116–125. https://doi.org/10.1080/2314808X.2020.1769913

Alkarithi, G., Duval, C., Shi, Y., Macrae, F.L., Ariëns, R.A.S., 2021. Thrombus Structural Composition in Cardiovascular Disease. Arteriosclerosis, Thrombosis, and Vascular Biology 41, 2370–2383. https://doi.org/10.1161/ATVBAHA.120.315754

Badeau, M., Adlercreutz, H., Kaihovaara, P., Tikkanen, M.J., 2005. Estrogen A-ring structure and antioxidative effect on lipoproteins. The Journal of Steroid Biochemistry and Molecular Biology 96, 271–278. https://doi.org/10.1016/j.jsbmb.2005.04.034

Balsano, F., Rizzon, P., Violi, F., Scrutinio, D., Cimminiello, C., Aguglia, F., Pasotti, C., Rudelli, G., 1990. Antiplatelet treatment with ticlopidine in unstable angina. A controlled multicenter clinical trial. The Studio della Ticlopidina nell'Angina Instabile Group. Circulation 82, 17–26. https://doi.org/10.1161/01.cir.82.1.17

Barton, M., Meyer, M.R., 2009. Postmenopausal hypertension: mechanisms and therapy. Hypertension 54, 11–18. https://doi.org/10.1161/HYPERTENSIONAHA.108.120022

Batty, P., Smith, J.G., 2010. Haemostasis. Surgery (Oxford), Emergency Surgery 28, 530–535. https://doi.org/10.1016/j.mpsur.2010.08.008

Bedell, S., Nachtigall, M., Naftolin, F., 2014. The pros and cons of plant estrogens for menopause. The Journal of Steroid Biochemistry and Molecular Biology 139, 225–236. https://doi.org/10.1016/j.jsbmb.2012.12.004

Bejma, J., Ji, L.L., 1999. Aging and acute exercise enhance free radical generation in rat skeletal muscle. J Appl Physiol (1985) 87, 465–470. https://doi.org/10.1152/jappl.1999.87.1.465

Bergstrom, J., 1975. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scand J Clin Lab Invest 35, 609–616.

Bertrand, M.E., Rupprecht, H.J., Urban, P., Gershlick, A.H., CLASSICS Investigators, 2000. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting : the clopidogrel aspirin stent international cooperative study (CLASSICS). Circulation 102, 624–629. https://doi.org/10.1161/01.cir.102.6.624

Bittner, V., 2009. Menopause, Age, and Cardiovascular Risk. Journal of the American College of Cardiology 54, 2374–2375. https://doi.org/10.1016/j.jacc.2009.10.008

Bossavy, J.-P., Thalamas, C., Sagnard, L., Barret, A., Sakariassen, K., Boneu, B., Cadroy, Y., 1998. A Double-Blind Randomized Comparison of Combined Aspirin and Ticlopidine Therapy Versus Aspirin or Ticlopidine Alone on Experimental Arterial

Thrombogenesis in Humans. Blood 92, 1518–1525. https://doi.org/10.1182/blood.V92.5.1518
Bouchard, C., An, P., Rice, T., Skinner, J.S., Wilmore, J.H., Gagnon, J., Pérusse, L., Leon, A.S., Rao, D.C., 1999. Familial aggregation ofV˙o 2 max response to exercise training: results from the HERITAGE Family Study. Journal of Applied Physiology 87, 1003–1008. https://doi.org/10.1152/jappl.1999.87.3.1003
Bourgeois, F.T., Orenstein, L., Ballakur, S., Mandl, K.D., Ioannidis, J.P.A., 2017. Exclusion of Elderly People from Randomized Clinical Trials of Drugs for Ischemic Heart Disease. J Am Geriatr Soc 65, 2354–2361. https://doi.org/10.1111/jgs.14833
Brass, L.F., 2003. Thrombin and Platelet Activation. Chest 124, 18S-25S. https://doi.org/10.1378/chest.124.3_suppl.18S
Breet, N.J., Sluman, M.A., van Berkel, M.A.J.P.J., van Werkum, J.W., Bouman, H.J., Harmsze, A.M., Kelder, J.C., Zijlstra, F., Hackeng, C.M., ten Berg, J.M., 2011. Effect of gender difference on platelet reactivity. Neth Heart J 19, 451–457. https://doi.org/10.1007/s12471-011-0189-y
Briasoulis, A., Telila, T., Palla, M., Siasos, G., Tousoulis, D., 2016. P2Y12 Receptor Antagonists: Which One to Choose? A Systematic Review and Meta-Analysis. Curr Pharm Des 22, 4568–4576. https://doi.org/10.2174/1381612822666160608114424
Brown, R.G., Davidson, L.A.G., Mckeown, T., Whitfield, A.G.W., 1957. CORONARY-ARTERY DISEASE: INFLUENCES AFFECTING ITS INCIDENCE IN MALES IN THE SEVENTH DECADE. The Lancet, Originally published as Volume 2, Issue 7005 270, 1073–1077. https://doi.org/10.1016/S0140-6736(57)90113-7
Brownley, K.A., Hinderliter, A.L., West, S.G., Grewen, K.M., Steege, J.F., Girdler, S.S., Light, K.C., 2004. Cardiovascular effects of 6 months of hormone replacement therapy versus placebo: differences associated with years since menopause. American Journal of Obstetrics and Gynecology 190, 1052–1058. https://doi.org/10.1016/j.ajog.2003.09.045
Brzozowska, M., Lewiński, A., 2020. Changes of androgens levels in menopausal women. Prz Menopauzalny 19, 151–154. https://doi.org/10.5114/pm.2020.101941
Burger, H.G., 2002. Androgen production in women. Fertil Steril 77 Suppl 4, S3-5. https://doi.org/10.1016/s0015-0282(02)02985-0
Cauley, J.A., Robbins, J., Chen, Z., Cummings, S.R., Jackson, R.D., LaCroix, A.Z., LeBoff, M., Lewis, C.E., McGowan, J., Neuner, J., Pettinger, M., Stefanick, M.L., Wactawski-Wende, J., Watts, N.B., for the Women's Health Initiative Investigators, 2003. Effects of Estrogen Plus Progestin on Risk of Fracture and Bone Mineral DensityThe Women's Health Initiative Randomized Trial. JAMA 290, 1729–1738. https://doi.org/10.1001/jama.290.13.1729
Celermajer, D.S., Sorensen, K.E., Spiegelhalter, D.J., Georgakopoulos, D., Robinson, J., Deanfield, J.E., 1994. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol 24, 471–476. https://doi.org/10.1016/0735-1097(94)90305-0
Chan, M.V., Knowles, R.B.M., Lundberg, M.H., Tucker, A.T., Mohamed, N.A., Kirkby, N.S., Armstrong, P.C.J., Mitchell, J.A., Warner, T.D., 2016. P2Y12 receptor blockade

synergizes strongly with nitric oxide and prostacyclin to inhibit platelet activation. Br J Clin Pharmacol 81, 621–633. https://doi.org/10.1111/bcp.12826

Chapin, J.C., Hajjar, K.A., 2015. Fibrinolysis and the control of blood coagulation. Blood Rev 29, 17–24. https://doi.org/10.1016/j.blre.2014.09.003

Chlebowski, R.T., Wactawski-Wende, J., Ritenbaugh, C., Hubbell, F.A., Ascensao, J., Rodabough, R.J., Rosenberg, C.A., Taylor, V.M., Harris, R., Chen, C., Adams-Campbell, L.L., White, E., 2004. Estrogen plus Progestin and Colorectal Cancer in Postmenopausal Women. New England Journal of Medicine 350, 991–1004. https://doi.org/10.1056/NEJMoa032071

Cho, L., Mukherjee, D., 2005. Hormone replacement therapy and secondary cardiovascular prevention: a meta-analysis of randomized trials. Cardiology 104, 143–147. https://doi.org/10.1159/000087635

Chrzanowski-Smith, O.J., Piatrikova, E., Betts, J.A., Williams, S., Gonzalez, J.T., 2020. Variability in exercise physiology: Can capturing intra-individual variation help better understand true inter-individual responses? European Journal of Sport Science 20, 452–460. https://doi.org/10.1080/17461391.2019.1655100

Ciana, P., Raviscioni, M., Mussi, P., Vegeto, E., Que, I., Parker, M.G., Lowik, C., Maggi, A., 2003. In vivo imaging of transcriptionally active estrogen receptors. Nat Med 9, 82–86. https://doi.org/10.1038/nm809

Colacurci, N., Chiàntera, A., Fornaro, F., de Novellis, V., Manzella, D., Arciello, A., Chiàntera, V., Improta, L., Paolisso, G., 2005. Effects of soy isoflavones on endothelial function in healthy postmenopausal women. Menopause 12, 299–307. https://doi.org/10.1097/01.GME.0000147017.23173.5B

Collet, J.-P., Thiele, H., Barbato, E., Barthélémy, O., Bauersachs, J., Bhatt, D.L., Dendale, P., Dorobantu, M., Edvardsen, T., Folliguet, T., Gale, C.P., Gilard, M., Jobs, A., Jüni, P., Lambrinou, E., Lewis, B.S., Mehilli, J., Meliga, E., Merkely, B., Mueller, C., Roffi, M., Rutten, F.H., Sibbing, D., Siontis, G.C.M., ESC Scientific Document Group, Kastrati, A., Mamas, M.A., Aboyans, V., Angiolillo, D.J., Bueno, H., Bugiardini, R., Byrne, R.A., Castelletti, S., Chieffo, A., Cornelissen, V., Crea, F., Delgado, V., Drexel, H., Gierlotka, M., Halvorsen, S., Haugaa, K.H., Jankowska, E.A., Katus, H.A., Kinnaird, T., Kluin, J., Kunadian, V., Landmesser, U., Leclercq, C., Lettino, M., Meinila, L., Mylotte, D., Ndrepepa, G., Omerovic, E., Pedretti, R.F.E., Petersen, S.E., Petronio, A.S., Pontone, G., Popescu, B.A., Potpara, T., Ray, K.K., Luciano, F., Richter, D.J., Shlyakhto, E., Simpson, I.A., Sousa-Uva, M., Storey, R.F., Touyz, R.M., Valgimigli, M., Vranckx, P., Yeh, R.W., Barbato, E., Barthélémy, O., Bauersachs, J., Bhatt, D.L., Dendale, P., Dorobantu, M., Edvardsen, T., Folliguet, T., Gale, C.P., Gilard, M., Jobs, A., Jüni, P., Lambrinou, E., Lewis, B.S., Mehilli, J., Meliga, E., Merkely, B., Mueller, C., Roffi, M., Rutten, F.H., Sibbing, D., Siontis, G.C.M., 2021. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. European Heart Journal 42, 1289–1367. https://doi.org/10.1093/eurheartj/ehaa575

Costello, J.T., Bieuzen, F., Bleakley, C.M., 2014. Where are all the female participants in Sports and Exercise Medicine research? European Journal of Sport Science 14, 847–851. https://doi.org/10.1080/17461391.2014.911354

Couse, J.F., Lindzey, J., Grandien, K., Gustafsson, J.A., Korach, K.S., 1997. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 138, 4613–4621. https://doi.org/10.1210/endo.138.11.5496

Craige, S.M., Kröller-Schön, S., Li, C., Kant, S., Cai, S., Chen, K., Contractor, M.M., Pei, Y., Schulz, E., Keaney, J.F., 2016. PGC-1α dictates endothelial function through regulation of eNOS expression. Sci Rep 6, 38210. https://doi.org/10.1038/srep38210

Curtis, D.J., Brown, M.R., Hawkins, K., Evans, P.A., Lawrence, M.J., Rees, P., Williams, P.R., 2011. Rheometrical and molecular dynamics simulation studies of incipient clot formation in fibrin-thrombin gels: An activation limited aggregation approach. Journal of Non-Newtonian Fluid Mechanics, Papers Presented at the University of Wales Institute of Non-Newtonian Fluid Mechanics Meeting on Rheometry, 29-31 March 2010, Lake Vyrnwy, Wales 166, 932–938. https://doi.org/10.1016/j.jnnfm.2011.04.016

Curtis, D.J., Williams, P.R., Badiei, N., Campbell, A.I., Hawkins, K., Evans, P.A., Brown, M.R., 2013. A study of microstructural templating in fibrin–thrombin gel networks by spectral and viscoelastic analysis. Soft Matter 9, 4883–4889. https://doi.org/10.1039/C3SM50263E

Danforth, D.R., Arbogast, L.K., Mroueh, J., Kim, M.H., Kennard, E.A., Seifer, D.B., Friedman, C.I., 1998. Dimeric inhibin: a direct marker of ovarian aging. Fertil Steril 70, 119–123. https://doi.org/10.1016/s0015-0282(98)00127-7

Dastjerdi, M.S., Emami, T., Najafian, A., Amini, M., 2006. Mean platelet volume measurement, EDTA or citrate? Hematology 11, 317–319. https://doi.org/10.1080/10245330600954163

Davie, E.W., Ratnoff, O.D., 1964. Waterfall Sequence for Intrinsic Blood Clotting. Science 145, 1310–1312. https://doi.org/10.1126/science.145.3638.1310

Davies, N.A., Llwyd, O., Brugniaux, J.V., Davies, G.R., Marley, C.J., Hodson, D., Lawrence, M.J., D'Silva, L.A., Morris, R.H.K., Hawkins, K., Williams, P.R., Bailey, D.M., Evans, P.A., 2016. Effects of exercise intensity on clot microstructure and mechanical properties in healthy individuals. Thrombosis Research 143, 130–136. https://doi.org/10.1016/j.thromres.2016.05.018

DeSouza, C.A., Shapiro, L.F., Clevenger, C.M., Dinenno, F.A., Monahan, K.D., Tanaka, H., Seals, D.R., 2000. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 102, 1351–1357. https://doi.org/10.1161/01.cir.102.12.1351

Dhalla, N.S., Temsah, R.M., Netticadan, T., 2000. Role of oxidative stress in cardiovascular diseases. J Hypertens 18, 655–673. https://doi.org/10.1097/00004872-200018060-00002

Dobesh, P.P., Oestreich, J.H., 2014. Ticagrelor: Pharmacokinetics, Pharmacodynamics, Clinical Efficacy, and Safety. Pharmacotherapy 34, 1077–1090. https://doi.org/10.1002/phar.1477

Dorsam, R.T., Kunapuli, S.P., 2004. Central role of the $P2Y_{12}$ receptor in platelet activation. J Clin Invest 113, 340–345. https://doi.org/10.1172/JCI20986

Egelund, J., Jørgensen, P.G., Mandrup, C.M., Fritz-Hansen, T., Stallknecht, B., Bangsbo, J., Nyberg, M., Hellsten, Y., 2017. Cardiac Adaptations to High-Intensity Aerobic Training in Premenopausal and Recent Postmenopausal Women: The Copenhagen Women Study. J Am Heart Assoc 6, e005469. https://doi.org/10.1161/JAHA.117.005469

El Khoudary, S.R., Aggarwal, B., Beckie, T.M., Hodis, H.N., Johnson, A.E., Langer, R.D., Limacher, M.C., Manson, J.E., Stefanick, M.L., Allison, M.A., null, null, 2020. Menopause Transition and Cardiovascular Disease Risk: Implications for Timing of Early Prevention: A Scientific Statement From the American Heart Association. Circulation 142, e506–e532. https://doi.org/10.1161/CIR.0000000000000912

Elmariah, S., Mauri, L., Doros, G., Galper, B.Z., O'Neill, K.E., Steg, P.G., Kereiakes, D.J., Yeh, R.W., 2015. Extended duration dual antiplatelet therapy and mortality: a systematic review and meta-analysis. The Lancet 385, 792–798. https://doi.org/10.1016/S0140-6736(14)62052-3

Engbers, M.J., Van Hylckama Vlieg, A., Rosendaal, F.R., 2010. Venous thrombosis in the elderly: incidence, risk factors and risk groups. Journal of Thrombosis and Haemostasis 8, 2105–2112. https://doi.org/10.1111/j.1538-7836.2010.03986.x

Finkelstein, J.S., Brockwell, S.E., Mehta, V., Greendale, G.A., Sowers, M.R., Ettinger, B., Lo, J.C., Johnston, J.M., Cauley, J.A., Danielson, M.E., Neer, R.M., 2008. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J Clin Endocrinol Metab 93, 861–868. https://doi.org/10.1210/jc.2007-1876

FitzGerald, G.A., Oates, J.A., Hawiger, J., Maas, R.L., Roberts, L.J., Lawson, J.A., Brash, A.R., 1983. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest 71, 676–688. https://doi.org/10.1172/JCI110814

Fleming, I., 2010. Molecular mechanisms underlying the activation of eNOS. Pflugers Arch 459, 793–806. https://doi.org/10.1007/s00424-009-0767-7

Fogle, R.H., Stanczyk, F.Z., Zhang, X., Paulson, R.J., 2007. Ovarian androgen production in postmenopausal women. J Clin Endocrinol Metab 92, 3040–3043. https://doi.org/10.1210/jc.2007-0581

Förstermann, U., Münzel, T., 2006. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113, 1708–1714. https://doi.org/10.1161/CIRCULATIONAHA.105.602532

Fournier, A., Mesrine, S., Dossus, L., Boutron-Ruault, M.-C., Clavel-Chapelon, F., Chabbert-Buffet, N., 2014. Risk of breast cancer after stopping menopausal hormone therapy in the E3N cohort. Breast Cancer Res Treat 145, 535–543. https://doi.org/10.1007/s10549-014-2934-6

Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri, M., Salvioli, S., 2018. The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. Frontiers in Medicine 5.

Francis, A.A., Pierce, G.N., 2011. An integrated approach for the mechanisms responsible for atherosclerotic plaque regression. Exp Clin Cardiol 16, 77–86.

Fredette, N.C., Meyer, M.R., Prossnitz, E.R., 2018. Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. J Steroid Biochem Mol Biol 176, 65–72. https://doi.org/10.1016/j.jsbmb.2017.05.006

Fuentes, N., Silveyra, P., 2019. Estrogen receptor signaling mechanisms. Advances in protein chemistry and structural biology 116, 135. https://doi.org/10.1016/bs.apcsb.2019.01.001

Fuller, R., Chavez, B., 2012. Ticagrelor (Brilinta), an Antiplatelet Drug for Acute Coronary Syndrome. P T 37, 562–568.

Gale, A.J., 2011. Current Understanding of Hemostasis. Toxicol Pathol 39, 273–280. https://doi.org/10.1177/0192623310389474

García-Martínez, M.C., Hermenegildo, C., Tarín, J.J., Cano, A., 2003. Phytoestrogens increase the capacity of serum to stimulate prostacyclin release in human endothelial cells. Acta Obstet Gynecol Scand 82, 705–710. https://doi.org/10.1080/j.1600-0412.2003.00178.x

Gent, M., Blakely, J.A., Easton, J.D., Ellis, D.J., Hachinski, V.C., Harbison, J.W., Panak, E., Roberts, R.S., Sicurella, J., Turpie, A.G., 1989. The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. Lancet 1, 1215–1220. https://doi.org/10.1016/s0140-6736(89)92327-1

Gliemann, L., Hellsten, Y., 2019. The exercise timing hypothesis: can exercise training compensate for the reduction in blood vessel function after menopause if timed right? The Journal of Physiology 597, 4915–4925. https://doi.org/10.1113/JP277056

Gliemann, L., Rytter, N., Tamariz-Ellemann, A., Egelund, J., Brandt, N., Carter, H.H., Hellsten, Y., 2020. Lifelong Physical Activity Determines Vascular Function in Late Postmenopausal Women. Med Sci Sports Exerc 52, 627–636. https://doi.org/10.1249/MSS.0000000000002180

Goldman, J.A., 2004. The Women's Health Initiative 2004 - Review and Critique. MedGenMed 6, 65.

Government of Canada, 2021. Canadian Health Measures Survey: Activity monitor data, 2018-2019 [WWW Document]. URL https://www150.statcan.gc.ca/n1/daily-quotidien/210901/dq210901c-eng.htm (accessed 10.12.22).

Grady, D., Rubin, S.M., Petitti, D.B., Fox, C.S., Black, D., Ettinger, B., Ernster, V.L., Cummings, S.R., 1992. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 117, 1016–1037. https://doi.org/10.7326/0003-4819-117-12-1016

Green, D.J., Maiorana, A., O'Driscoll, G., Taylor, R., 2004. Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol 561, 1–25. https://doi.org/10.1113/jphysiol.2004.068197

Greendale, G.A., Reboussin, B.A., Hogan, P., Barnabei, V.M., Shumaker, S., Johnson, S., Barrett-Connor, E., 1998. Symptom relief and side effects of postmenopausal hormones: results from the postmenopausal estrogen/progestin interventions trial. Obstetrics & Gynecology 92, 982–988. https://doi.org/10.1016/S0029-7844(98)00305-6

Gregorini, L., Marco, J., 1997. Ticlopidine and aspirin interactions. Heart 77, 11–12. https://doi.org/10.1136/hrt.77.1.11

Grindler, N.M., Santoro, N.F., 2015. Menopause and exercise. Menopause 22, 1351–1358. https://doi.org/10.1097/GME.0000000000000536

Grodstein, F., Stampfer, M.J., Colditz, G.A., Willett, W.C., Manson, J.E., Joffe, M., Rosner, B., Fuchs, C., Hankinson, S.E., Hunter, D.J., Hennekens, C.H., Speizer, F.E., 1997. Postmenopausal Hormone Therapy and Mortality. New England Journal of Medicine 336, 1769–1776. https://doi.org/10.1056/NEJM199706193362501

Hackley, B., Rousseau, M.E., 2004. Managing menopausal symptoms after the women's health initiative. Journal of Midwifery & Women's Health 49, 87–95. https://doi.org/10.1016/j.jmwh.2003.12.005

Hall, W.L., Vafeiadou, K., Hallund, J., Bügel, S., Koebnick, C., Reimann, M., Ferrari, M., Branca, F., Talbot, D., Dadd, T., Nilsson, M., Dahlman-Wright, K., Gustafsson, J.-A., Minihane, A.-M., Williams, C.M., 2005. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. Am J Clin Nutr 82, 1260–1268; quiz 1365–1366. https://doi.org/10.1093/ajcn/82.6.1260

Hamilos, M., Petousis, S., Parthenakis, F., 2018. Interaction between platelets and endothelium: from pathophysiology to new therapeutic options. Cardiovasc Diagn Ther 8, 568–580. https://doi.org/10.21037/cdt.2018.07.01

Hamilton, K.J., Hewitt, S.C., Arao, Y., Korach, K.S., 2017. Estrogen Hormone Biology. Curr Top Dev Biol 125, 109–146. https://doi.org/10.1016/bs.ctdb.2016.12.005

Hanby, H.A., Bao, J., Noh, J.-Y., Jarocha, D., Poncz, M., Weiss, M.J., Marks, M.S., 2017. Platelet dense granules begin to selectively accumulate mepacrine during proplatelet formation. Blood Advances 1, 1478–1490. https://doi.org/10.1182/bloodadvances.2017006726

Haney, A., Wild, R., 2007. Options for hormone therapy in women who have had a hysterectomy. Menopause (New York, N.Y.) 14. https://doi.org/10.1097/gme.0b013e31804154d5

Harlow, S.D., Mitchell, E.S., Crawford, S., Nan, B., Little, R., Taffe, J., ReSTAGE Collaboration, 2008. The ReSTAGE Collaboration: defining optimal bleeding criteria for onset of early menopausal transition. Fertil Steril 89, 129–140. https://doi.org/10.1016/j.fertnstert.2007.02.015

Harper-Harrison, G., Shanahan, M.M., 2022. Hormone Replacement Therapy, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Harridge, S.D.R., Lazarus, N.R., 2017. Physical Activity, Aging, and Physiological Function. Physiology (Bethesda) 32, 152–161. https://doi.org/10.1152/physiol.00029.2016

Harris, G.W., 1948. Electrical stimulation of the hypothalamus and the mechanism of neural control of the adenohypophysis. J Physiol 107, 418–429. https://doi.org/10.1113/jphysiol.1948.sp004286

Hass, W.K., Easton, J.D., Adams, H.P., Pryse-Phillips, W., Molony, B.A., Anderson, S., Kamm, B., 1989. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. Ticlopidine Aspirin Stroke Study Group. N Engl J Med 321, 501–507. https://doi.org/10.1056/NEJM198908243210804

Hawley, J.A., Burke, L.M., Phillips, S.M., Spriet, L.L., 2011. Nutritional modulation of training-induced skeletal muscle adaptations. J Appl Physiol (1985) 110, 834–845. https://doi.org/10.1152/japplphysiol.00949.2010

He, F., Li, J., Liu, Z., Chuang, C.-C., Yang, W., Zuo, L., 2016. Redox Mechanism of Reactive Oxygen Species in Exercise. Front Physiol 7, 486. https://doi.org/10.3389/fphys.2016.00486

Hecker, M., Ullrich, V., 1989. On the mechanism of prostacyclin and thromboxane A2 biosynthesis. J Biol Chem 264, 141–150.

Henderson, B.E., Paganini-Hill, A., Ross, R.K., 1991. Decreased Mortality in Users of Estrogen Replacement Therapy. Archives of Internal Medicine 151, 75–78. https://doi.org/10.1001/archinte.1991.00400010095012

Hiller, E., 2007. Basic Principles of Hemostasis, in: Munker, R., Hiller, E., Glass, J., Paquette, R. (Eds.), Modern Hematology: Biology and Clinical Management, Contemporary Hematology. Humana Press, Totowa, NJ, pp. 327–345. https://doi.org/10.1007/978-1-59745-149-9_19

Hinchliff, S., 2019. Menopause, in: Gu, D., Dupre, M.E. (Eds.), Encyclopedia of Gerontology and Population Aging. Springer International Publishing, Cham, pp. 1–5. https://doi.org/10.1007/978-3-319-69892-2_161-1

Hodis, H.N., Mack, W.J., Henderson, V.W., Shoupe, D., Budoff, M.J., Hwang-Levine, J., Li, Y., Feng, M., Dustin, L., Kono, N., Stanczyk, F.Z., Selzer, R.H., Azen, S.P., 2016. Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol. N Engl J Med 374, 1221–1231. https://doi.org/10.1056/NEJMoa1505241

Hoffman, B.B., Michel, T., Brenneman, T.B., Lefkowitz, R.J., 1982. Interactions of agonists with platelet alpha 2-adrenergic receptors. Endocrinology 110, 926–932. https://doi.org/10.1210/endo-110-3-926

Hoier, B., Nordsborg, N., Andersen, S., Jensen, L., Nybo, L., Bangsbo, J., Hellsten, Y., 2012. Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. The Journal of Physiology 590, 595–606. https://doi.org/10.1113/jphysiol.2011.216135

Hoier, B., Olsen, L.N., Leinum, M., Jørgensen, T.S., Carter, H.H., Hellsten, Y., Bangsbo, J., 2021. Aerobic High-Intensity Exercise Training Improves Cardiovascular Health in Older Post-menopausal Women. Front Aging 2, 667519. https://doi.org/10.3389/fragi.2021.667519

Holder, S.M., Brislane, Á., Dawson, E.A., Hopkins, N.D., Hopman, M.T.E., Cable, N.T., Jones, H., Schreuder, T.H.A., Sprung, V.S., Naylor, L., Maiorana, A., Thompson, A., Thijssen, D.H.J., Green, D.J., 2019. Relationship Between Endothelial Function

and the Eliciting Shear Stress Stimulus in Women: Changes Across the Lifespan Differ to Men. J Am Heart Assoc 8, e010994. https://doi.org/10.1161/JAHA.118.010994

Horn-Ross, P.L., John, E.M., Canchola, A.J., Stewart, S.L., Lee, M.M., 2003. Phytoestrogen intake and endometrial cancer risk. J Natl Cancer Inst 95, 1158–1164. https://doi.org/10.1093/jnci/djg015

Horstman, A.M., Dillon, E.L., Urban, R.J., Sheffield-Moore, M., 2012. The Role of Androgens and Estrogens on Healthy Aging and Longevity. J Gerontol A Biol Sci Med Sci 67, 1140–1152. https://doi.org/10.1093/gerona/gls068

Howes, J.B., Tran, D., Brillante, D., Howes, L.G., 2003. Effects of dietary supplementation with isoflavones from red clover on ambulatory blood pressure and endothelial function in postmenopausal type 2 diabetes. Diabetes Obes Metab 5, 325–332. https://doi.org/10.1046/j.1463-1326.2003.00282.x

Hsia, J., Langer, R.D., Manson, J.E., Kuller, L., Johnson, K.C., Hendrix, S.L., Pettinger, M., Heckbert, S.R., Greep, N., Crawford, S., Eaton, C.B., Kostis, J.B., Caralis, P., Prentice, R., Women's Health Initiative Investigators, 2006. Conjugated equine estrogens and coronary heart disease: the Women's Health Initiative. Arch Intern Med 166, 357–365. https://doi.org/10.1001/archinte.166.3.357

Huveneers, S., Daemen, M.J.A.P., Hordijk, P.L., 2015. Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. Circ Res 116, 895–908. https://doi.org/10.1161/CIRCRESAHA.116.305720

Hyde, E.T., Whitfield, G.P., Omura, J.D., Fulton, J.E., Carlson, S.A., 2021. Trends in Meeting the Physical Activity Guidelines: Muscle-Strengthening Alone and Combined With Aerobic Activity, United States, 1998–2018. Journal of Physical Activity and Health 18, S37–S44. https://doi.org/10.1123/jpah.2021-0077

Iqbal, J., Zaidi, M., 2009. Understanding Estrogen Action during Menopause. Endocrinology 150, 3443–3445. https://doi.org/10.1210/en.2009-0449

Izquierdo, M., Häkkinen, K., Antón, A., Garrues, M., Ibañez, J., Ruesta, M., Gorostiaga, E.M., 2001. Maximal strength and power, endurance performance, and serum hormones in middle-aged and elderly men. Med Sci Sports Exerc 33, 1577–1587. https://doi.org/10.1097/00005768-200109000-00022

Izzo, C., Vitillo, P., Di Pietro, P., Visco, V., Strianese, A., Virtuoso, N., Ciccarelli, M., Galasso, G., Carrizzo, A., Vecchione, C., 2021. The Role of Oxidative Stress in Cardiovascular Aging and Cardiovascular Diseases. Life (Basel) 11, 60. https://doi.org/10.3390/life11010060

Jin, J., Daniel, J.L., Kunapuli, S.P., 1998. Molecular basis for ADP-induced platelet activation. II. The P2Y1 receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. J Biol Chem 273, 2030–2034. https://doi.org/10.1074/jbc.273.4.2030

Jones, S.P., Bolli, R., 2006. The ubiquitous role of nitric oxide in cardioprotection. Journal of Molecular and Cellular Cardiology 40, 16–23. https://doi.org/10.1016/j.yjmcc.2005.09.011

Joo, J., Williamson, S.A., Vazquez, A.I., Fernandez, J.R., Bray, M.S., 2019. The influence of 15-week exercise training on dietary patterns among young adults. Int J Obes 43, 1681–1690. https://doi.org/10.1038/s41366-018-0299-3

Joswig, M., Hach-Wunderle, V., von Holst, T., Nawroth, P.P., 2000. Postmenopausal hormone replacement therapy and the vascular wall: epidemiology and clinical studies. Vasa 29, 243–251. https://doi.org/10.1024/0301-1526.29.4.243

Kalhan, M., Singhania, K., Choudhary, P., Verma, S., Kaushal, P., Singh, T., 2020. Prevalence of Menopausal Symptoms and its Effect on Quality of Life among Rural Middle Aged Women (40–60 Years) of Haryana, India. Int J Appl Basic Med Res 10, 183–188. https://doi.org/10.4103/ijabmr.IJABMR_428_19

Kerlikowske, K., Miglioretti, D.L., Ballard-Barbash, R., Weaver, D.L., Buist, D.S.M., Barlow, W.E., Cutter, G., Geller, B.M., Yankaskas, B., Taplin, S.H., Carney, P.A., 2003. Prognostic Characteristics of Breast Cancer Among Postmenopausal Hormone Users in a Screened Population. JCO 21, 4314–4321. https://doi.org/10.1200/JCO.2003.05.151

Kim, M.R., Kim, H.J., Yu, S.H., Lee, B.S., Jeon, S.Y., Lee, J.J., Lee, Y.C., 2020. Combination of Red Clover and Hops Extract Improved Menopause Symptoms in an Ovariectomized Rat Model. Evid Based Complement Alternat Med 2020, 7941391. https://doi.org/10.1155/2020/7941391

Kim, Y., Lee, E., 2019. The association between elderly people's sedentary behaviors and their health-related quality of life: focusing on comparing the young-old and the old-old. Health and Quality of Life Outcomes 17, 131. https://doi.org/10.1186/s12955-019-1191-0

Kirkby, N.S., Lundberg, M.H., Chan, M.V., Vojnovic, I., Solomon, A.B., Emerson, M., Mitchell, J.A., Warner, T.D., 2013. Blockade of the purinergic P2Y12 receptor greatly increases the platelet inhibitory actions of nitric oxide. Proc Natl Acad Sci U S A 110, 15782–15787. https://doi.org/10.1073/pnas.1218880110

Koenig, A., Buskiewicz, I., Huber, S.A., 2017. Age-Associated Changes in Estrogen Receptor Ratios Correlate with Increased Female Susceptibility to Coxsackievirus B3-Induced Myocarditis. Front Immunol 8, 1585. https://doi.org/10.3389/fimmu.2017.01585

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.A., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870. https://doi.org/10.1210/endo.138.3.4979

Lablanche, J.M., McFadden, E.P., Bonnet, J.L., Grollier, G., Danchin, N., Bedossa, M., Leclercq, C., Vahanian, A., Bauters, C., Van Belle, E., Bertrand, M.E., 1996. Combined antiplatelet therapy with ticlopidine and aspirin. A simplified approach to intracoronary stent management. Eur Heart J 17, 1373–1380. https://doi.org/10.1093/oxfordjournals.eurheartj.a015072

Laganière, J., Tremblay, G.B., Dufour, C.R., Giroux, S., Rousseau, F., Giguère, V., 2004. A polymorphic autoregulatory hormone response element in the human estrogen-related receptor alpha (ERRalpha) promoter dictates peroxisome proliferator-

activated receptor gamma coactivator-1alpha control of ERRalpha expression. J Biol Chem 279, 18504–18510. https://doi.org/10.1074/jbc.M313543200
Lawrence, M.J., Davies, G., Nyberg, M., Whitley, J., Evans, V., Williams, R., Hellsten, Y., Evans, P.A., 2018. The effect of tyramine infusion and exercise on blood flow, coagulation and clot microstructure in healthy individuals. Thrombosis Research 170, 32–37. https://doi.org/10.1016/j.thromres.2018.07.025
Lawrence, M.J., Kumar, S., Hawkins, K., Boden, S., Rutt, H., Mills, G., Sabra, A., Morris, R.H.K., Davidson, S.J., Badiei, N., Brown, M.R., Williams, P.R., Evans, P.A., 2014. A new structural biomarker that quantifies and predicts changes in clot strength and quality in a model of progressive haemodilution. Thrombosis Research 134, 488–494. https://doi.org/10.1016/j.thromres.2014.05.039
Lazarus, N.R., Harridge, S.D.R., 2017. Declining performance of master athletes: silhouettes of the trajectory of healthy human ageing? The Journal of Physiology 595, 2941–2948. https://doi.org/10.1113/JP272443
Le Blanc, J., Lordkipanidzé, M., 2019. Platelet Function in Aging. Front Cardiovasc Med 6, 109. https://doi.org/10.3389/fcvm.2019.00109
Lebon, J., Riesco, E., Tessier, D., Dionne, I.J., 2014. Additive effects of isoflavones and exercise training on inflammatory cytokines and body composition in overweight and obese postmenopausal women: a randomized controlled trial. Menopause 21, 869–875. https://doi.org/10.1097/GME.0000000000000177
Lecut, C., Schoolmeester, A., Kuijpers, M.J.E., Broers, J.L.V., van Zandvoort, M.A.M.J., Vanhoorelbeke, K., Deckmyn, H., Jandrot-Perrus, M., Heemskerk, J.W.M., 2004. Principal role of glycoprotein VI in alpha2beta1 and alphaIIbbeta3 activation during collagen-induced thrombus formation. Arterioscler Thromb Vasc Biol 24, 1727–1733. https://doi.org/10.1161/01.ATV.0000137974.85068.93
Lee, H.-L., Kim, J.-T., Lee, J.S., Park, M.-S., Choi, K.-H., Cho, K.-H., Kim, B.J., Park, J.-M., Kang, K., Lee, S.J., Kim, J.G., Cha, J.-K., Kim, D.-H., Park, T.H., Park, S.-S., Lee, K.B., Lee, Jun, Hong, K.-S., Cho, Y.-J., Park, H.-K., Lee, B.-C., Yu, K.-H., Sun Oh, M., Kim, D.-E., Ryu, W.-S., Choi, J.C., Kwon, J.-H., Kim, W.-J., Shin, D.-I., Sohn, S.I., Hong, J.-H., Lee, Juneyoung, Bae, H.-J., 2020. Comparative Effectiveness of Dual Antiplatelet Therapy With Aspirin and Clopidogrel Versus Aspirin Monotherapy in Mild-to-Moderate Acute Ischemic Stroke According to the Risk of Recurrent Stroke. Circulation: Cardiovascular Quality and Outcomes 13, e006474. https://doi.org/10.1161/CIRCOUTCOMES.119.006474
Lemežienė, N., Padarauskas, A., Butkutė, B., Cesevičienė, J., Taujenis, L., Norkevičienė, E., Mikaliūnienė, J., 2015. The concentration of isoflavones in red clover (Trifolium pratense L.) at flowering stage. Žemdirbystė (Agriculture) 102, 443–448.
Levick, J.R., 1991. Chapter 1 - Overview of the cardiovascular system, in: Levick, J.R. (Ed.), An Introduction to Cardiovascular Physiology. Butterworth-Heinemann, pp. 1–12. https://doi.org/10.1016/B978-0-7506-1028-5.50004-4
Levin, E.R., 2009. Plasma Membrane Estrogen Receptors. Trends Endocrinol Metab 20, 477–482. https://doi.org/10.1016/j.tem.2009.06.009

Li, B., Wang, J.H.-C., 2011. Fibroblasts and Myofibroblasts in Wound Healing: Force Generation and Measurement. J Tissue Viability 20, 108–120. https://doi.org/10.1016/j.jtv.2009.11.004
Li, C.I., Malone, K.E., Porter, P.L., Weiss, N.S., Tang, M.-T.C., Cushing-Haugen, K.L., Daling, J.R., 2003. Relationship Between Long Durations and Different Regimens of Hormone Therapy and Risk of Breast Cancer. JAMA 289, 3254–3263. https://doi.org/10.1001/jama.289.24.3254
Li, S.-H., Liu, X.-X., Bai, Y.-Y., Wang, X.-J., Sun, K., Chen, J.-Z., Hui, R.-T., 2010. Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. Am J Clin Nutr 91, 480–486. https://doi.org/10.3945/ajcn.2009.28203
Li, T.Y., Rana, J.S., Manson, J.E., Willett, W.C., Stampfer, M.J., Colditz, G.A., Rexrode, K.M., Hu, F.B., 2006. Obesity as Compared With Physical Activity in Predicting Risk of Coronary Heart Disease in Women. Circulation 113, 499–506. https://doi.org/10.1161/CIRCULATIONAHA.105.574087
Li, Z., Delaney, M.K., O'Brien, K.A., Du, X., 2010. Signaling during platelet adhesion and activation. Arterioscler Thromb Vasc Biol 30, 2341–2349. https://doi.org/10.1161/ATVBAHA.110.207522
Lievertz, R.W., 1987. Pharmacology and pharmacokinetics of estrogens. American Journal of Obstetrics and Gynecology 156, 1289–1293. https://doi.org/10.1016/0002-9378(87)90166-9
Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. Clin Interv Aging 13, 757–772. https://doi.org/10.2147/CIA.S158513
Liu, D., Zhang, Z., Gladwell, W., Teng, C.T., 2003. Estrogen Stimulates Estrogen-Related Receptor α Gene Expression through Conserved Hormone Response Elements. Endocrinology 144, 4894–4904. https://doi.org/10.1210/en.2003-0432
Liu, Y., Ma, L., Yang, X., Bie, J., Li, D., Sun, C., Zhang, J., Meng, Y., Lin, J., 2019. Menopausal Hormone Replacement Therapy and the Risk of Ovarian Cancer: A Meta-Analysis. Frontiers in Endocrinology 10.
Lundberg Slingsby, M.H., Gliemann, L., Thrane, M., Rytter, N., Egelund, J., Chan, M.V., Armstrong, P.C., Warner, T.D., Hellsten, Y., 2018. Platelet responses to pharmacological and physiological interventions in middle-aged men with different habitual physical activity levels. Acta Physiol 223, e13028. https://doi.org/10.1111/apha.13028
Lundberg Slingsby, M.H., Nyberg, M., Egelund, J., Mandrup, C.M., Frikke-Schmidt, R., Kirkby, N.S., Hellsten, Y., 2017. Aerobic exercise training lowers platelet reactivity and improves platelet sensitivity to prostacyclin in pre- and postmenopausal women. J Thromb Haemost 15, 2419–2431. https://doi.org/10.1111/jth.13866
Macfarlane, R.G., 1964. An Enzyme Cascade in the Blood Clotting Mechanism, and its Function as a Biochemical Amplifier. Nature 202, 498–499. https://doi.org/10.1038/202498a0
Mackman, N., 2009. The Role of Tissue Factor and Factor VIIa in Hemostasis. Anesth Analg 108, 1447–1452. https://doi.org/10.1213/ane.0b013e31819bceb1

Maessen, M.F.H., Verbeek, A.L.M., Bakker, E.A., Thompson, P.D., Hopman, M.T.E., Eijsvogels, T.M.H., 2016. Lifelong Exercise Patterns and Cardiovascular Health. Mayo Clinic Proceedings 91, 745–754. https://doi.org/10.1016/j.mayocp.2016.02.028

Magnette, A., Chatelain, M., Chatelain, B., Ten Cate, H., Mullier, F., 2016. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. Thromb J 14, 49. https://doi.org/10.1186/s12959-016-0123-z

Mainini, G., Torella, M., Di Donna, M.C., Esposito, E., Ercolano, S., Correa, R., Cucinella, G., Stradella, L., Luisi, A., Basso, A., Cerreto, F.V., Cicatiello, R., Matteo, M., De Franciscis, P., 2013. Nonhormonal management of postmenopausal women: effects of a red clover based isoflavones supplementation on climacteric syndrome and cardiovascular risk serum profile. Clin Exp Obstet Gynecol 40, 337–341.

Mallidi, J., Lata, K., 2019. Role of Gender in Dual Antiplatelet Therapy After Acute Coronary Syndrome. Curr Atheroscler Rep 21, 34. https://doi.org/10.1007/s11883-019-0795-5

Mandrup, C.M., Egelund, J., Nyberg, M., Slingsby, M.H.L., Andersen, C.B., Løgstrup, S., Bangsbo, J., Suetta, C., Stallknecht, B., Hellsten, Y., 2017. Effects of high-intensity training on cardiovascular risk factors in premenopausal and postmenopausal women. American Journal of Obstetrics & Gynecology 216, 384.e1-384.e11. https://doi.org/10.1016/j.ajog.2016.12.017

Mann, T.N., Lamberts, R.P., Lambert, M.I., 2014. High responders and low responders: factors associated with individual variation in response to standardized training. Sports Med 44, 1113–1124. https://doi.org/10.1007/s40279-014-0197-3

Manolis, A.S., Manolis, T.A., Papadimitriou, P., Koulouris, S., Melita, H., 2013. Combined antiplatelet therapy: still a sweeping combination in cardiology. Cardiovasc Hematol Agents Med Chem 11, 136–167. https://doi.org/10.2174/1871525711311020009

Mari, D., Ogliari, G., Castaldi, D., Vitale, G., Bollini, E.M., Lio, D., 2008. Hemostasis and ageing. Immun Ageing 5, 12. https://doi.org/10.1186/1742-4933-5-12

Markopoulos, M.C., Kassi, E., Alexandraki, K.I., Mastorakos, G., Kaltsas, G., 2015. Hyperandrogenism after menopause. Eur J Endocrinol 172, R79-91. https://doi.org/10.1530/EJE-14-0468

Marrocco, J., McEwen, B.S., 2016. Sex in the brain: hormones and sex differences. Dialogues Clin Neurosci 18, 373–383.

Marsden, J., 2002. The menopause, hormone replacement therapy and breast cancer. The Journal of Steroid Biochemistry and Molecular Biology, Proceedings of the 15th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology “Recent Advances in Steroid Biochemistry and Molecular Biology” 83, 123–132. https://doi.org/10.1016/S0960-0760(02)00258-3

Matthews, K.A., Crawford, S.L., Chae, C.U., Everson-Rose, S.A., Sowers, M.F., Sternfeld, B., Sutton-Tyrrell, K., 2009. Are Changes in Cardiovascular Disease Risk Factors in Midlife Women due to Chronological Aging or to the Menopausal Transition? J Am Coll Cardiol 54, 2366–2373. https://doi.org/10.1016/j.jacc.2009.10.009

Mauri, L., Kereiakes, D.J., Yeh, R.W., Driscoll-Shempp, P., Cutlip, D.E., Steg, P.G., Normand, S.-L.T., Braunwald, E., Wiviott, S.D., Cohen, D.J., Holmes, D.R., Krucoff, M.W., Hermiller, J., Dauerman, H.L., Simon, D.I., Kandzari, D.E., Garratt, K.N., Lee, D.P., Pow, T.K., Ver Lee, P., Rinaldi, M.J., Massaro, J.M., 2014. Twelve or 30 Months of Dual Antiplatelet Therapy after Drug-Eluting Stents. New England Journal of Medicine 371, 2155–2166. https://doi.org/10.1056/NEJMoa1409312

McDonnell, D.P., Norris, J.D., 2002. Connections and Regulation of the Human Estrogen Receptor. Science 296, 1642–1644. https://doi.org/10.1126/science.1071884

Mercado, C.P., Kilic, F., 2010. Molecular Mechanisms of SERT in Platelets: Regulation of Plasma Serotonin Levels. Mol Interv 10, 231–241. https://doi.org/10.1124/mi.10.4.6

Merten, M., Chow, T., Hellums, J.D., Thiagarajan, P., 2000. A new role for P-selectin in shear-induced platelet aggregation. Circulation 102, 2045–2050. https://doi.org/10.1161/01.cir.102.17.2045

Merten, M., Pakala, R., Thiagarajan, P., Benedict, C.R., 1999. Platelet microparticles promote platelet interaction with subendothelial matrix in a glycoprotein IIb/IIIa-dependent mechanism. Circulation 99, 2577–2582. https://doi.org/10.1161/01.cir.99.19.2577

Miller, V.M., Black, D.M., Brinton, E.A., Budoff, M.J., Cedars, M.I., Hodis, H.N., Lobo, R.A., Manson, J.E., Merriam, G.R., Naftolin, F., Santoro, N., Taylor, H.S., Harman, S.M., 2009. Using basic science to design a clinical trial: baseline characteristics of women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). J Cardiovasc Transl Res 2, 228–239. https://doi.org/10.1007/s12265-009-9104-y

Miller, V.M., Duckles, S.P., 2008. Vascular Actions of Estrogens: Functional Implications. Pharmacological Reviews 60, 210–241. https://doi.org/10.1124/pr.107.08002

Miner, J., Hoffhines, A., 2007. The Discovery of Aspirin's Antithrombotic Effects. Tex Heart Inst J 34, 179–186.

Mitchell, J.A., Ali, F., Bailey, L., Moreno, L., Harrington, L.S., 2008. Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. Exp Physiol 93, 141–147. https://doi.org/10.1113/expphysiol.2007.038588

Mitchell, M.D., 1978. A sensitive radioimmunoassay for 6-keto-prostaglandin F1α: preliminary observations on circulating concentrations. Prostaglandins and Medicine 1, 13–21. https://doi.org/10.1016/0161-4630(78)90072-1

Montero, D., Lundby, C., 2017. Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. J Physiol 595, 3377–3387. https://doi.org/10.1113/JP273480

Moreau, K.L., Hildreth, K.L., 2014. Vascular Aging across the Menopause Transition in Healthy Women. Advances in Vascular Medicine 2014, 1–12. https://doi.org/10.1155/2014/204390

Moreau, K.L., Stauffer, B.L., Kohrt, W.M., Seals, D.R., 2013. Essential Role of Estrogen for Improvements in Vascular Endothelial Function With Endurance Exercise in Postmenopausal Women. J Clin Endocrinol Metab 98, 4507–4515. https://doi.org/10.1210/jc.2013-2183

Muscella, A., Stefàno, E., Marsigliante, S., 2020. The effects of exercise training on lipid metabolism and coronary heart disease. Am J Physiol Heart Circ Physiol 319, H76–H88. https://doi.org/10.1152/ajpheart.00708.2019

Naci, H., Ioannidis, J.P.A., 2013. Comparative effectiveness of exercise and drug interventions on mortality outcomes: metaepidemiological study. BMJ : British Medical Journal (Online) 347. https://doi.org/10.1136/bmj.f5577

Nadal, A., Díaz, M., Valverde, M.A., 2001. The Estrogen Trinity: Membrane, Cytosolic, and Nuclear Effects. Physiology 16, 251–255. https://doi.org/10.1152/physiologyonline.2001.16.6.251

Nauman, J., Tauschek, L.C., Kaminsky, L.A., Nes, B.M., Wisløff, U., 2017. Global Fitness Levels: Findings From a Web-Based Surveillance Report. Progress in Cardiovascular Diseases, Physical Activity, Exercise and Fitness in Health and Disease 60, 78–88. https://doi.org/10.1016/j.pcad.2017.01.009

Nelson, H.D., Humphrey, L.L., Nygren, P., Teutsch, S.M., Allan, J.D., 2002. Postmenopausal hormone replacement therapy: scientific review. JAMA 288, 872–881. https://doi.org/10.1001/jama.288.7.872

Nestel, P.J., Pomeroy, S., Kay, S., Komesaroff, P., Behrsing, J., Cameron, J.D., West, L., 1999. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. J Clin Endocrinol Metab 84, 895–898. https://doi.org/10.1210/jcem.84.3.5561

Nishiyama, S.K., Wray, D.W., Richardson, R.S., 2008. Aging affects vascular structure and function in a limb-specific manner. J Appl Physiol (1985) 105, 1661–1670. https://doi.org/10.1152/japplphysiol.90612.2008

Norgard, N.B., Abu-Fadel, M., 2009. Comparison of prasugrel and clopidogrel in patients with acute coronary syndrome undergoing percutaneous coronary intervention. Vasc Health Risk Manag 5, 873–882.

Novella, S., Heras, M., Hermenegildo, C., Dantas, A.P., 2012. Effects of Estrogen on Vascular Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology 32, 2035–2042. https://doi.org/10.1161/ATVBAHA.112.250308

Novensà, L., Novella, S., Medina, P., Segarra, G., Castillo, N., Heras, M., Hermenegildo, C., Dantas, A.P., 2011. Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ERα/ERβ balance in female mice. PLoS One 6, e25335. https://doi.org/10.1371/journal.pone.0025335

Nyberg, M., Blackwell, J.R., Damsgaard, R., Jones, A.M., Hellsten, Y., Mortensen, S.P., 2012. Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans. The Journal of Physiology 590, 5361–5370. https://doi.org/10.1113/jphysiol.2012.239053

Nyberg, M., Egelund, J., Mandrup, C.M., Andersen, C.B., Hansen, K.M.B.E., Hergel, I.-M.F., Valbak-Andersen, N., Frikke-Schmidt, R., Stallknecht, B., Bangsbo, J., Hellsten, Y., 2017. Leg vascular and skeletal muscle mitochondrial adaptations to aerobic high-intensity exercise training are enhanced in the early postmenopausal phase. J Physiol 595, 2969–2983. https://doi.org/10.1113/JP273871

Nyberg, M., Egelund, J., Mandrup, C.M., Nielsen, M.B., Mogensen, A.S., Stallknecht, B., Bangsbo, J., Hellsten, Y., 2016. Early Postmenopausal Phase Is Associated With Reduced Prostacyclin-Induced Vasodilation That Is Reversed by Exercise Training. Hypertension 68, 1011–1020. https://doi.org/10.1161/HYPERTENSIONAHA.116.07866

Nyberg, M., Seidelin, K., Andersen, T.R., Overby, N.N., Hellsten, Y., Bangsbo, J., 2014. Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise training. Am J Physiol Regul Integr Comp Physiol 306, R510-517. https://doi.org/10.1152/ajpregu.00539.2013

Nystoriak, M.A., Bhatnagar, A., 2018. Cardiovascular Effects and Benefits of Exercise. Front Cardiovasc Med 5, 135. https://doi.org/10.3389/fcvm.2018.00135

Ojeda, S.R., Prevot, V., Heger, S., Lomniczi, A., Dziedzic, B., Mungenast, A., 2003. The neurobiology of female puberty. Horm Res 60 Suppl 3, 15–20. https://doi.org/10.1159/000074495

Olvera Lopez, E., Ballard, B.D., Jan, A., 2022. Cardiovascular Disease, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Ozemek, C., Laddu, D.R., Lavie, C.J., Claeys, H., Kaminsky, L.A., Ross, R., Wisloff, U., Arena, R., Blair, S.N., 2018. An Update on the Role of Cardiorespiratory Fitness, Structured Exercise and Lifestyle Physical Activity in Preventing Cardiovascular Disease and Health Risk. Progress in Cardiovascular Diseases, Advances in Echocardiography 61, 484–490. https://doi.org/10.1016/j.pcad.2018.11.005

Palta, S., Saroa, R., Palta, A., 2014. Overview of the coagulation system. Indian J Anaesth 58, 515–523. https://doi.org/10.4103/0019-5049.144643

Park, Y.-M., Pereira, R.I., Erickson, C.B., Swibas, T.A., Kang, C., Van Pelt, R.E., 2017. Time since menopause and skeletal muscle estrogen receptors, PGC-1α, and AMPK. Menopause 24, 815–823. https://doi.org/10.1097/GME.0000000000000829

Parker, B.A., Kalasky, M.J., Proctor, D.N., 2010. Evidence for sex differences in cardiovascular aging and adaptive responses to physical activity. Eur J Appl Physiol 110, 235–246. https://doi.org/10.1007/s00421-010-1506-7

Parker, W.A.E., Orme, R.C., Hanson, J., Stokes, H.M., Bridge, C.M., Shaw, P.A., Sumaya, W., Thorneycroft, K., Petrucci, G., Porro, B., Judge, H.M., Ajjan, R.A., Rocca, B., Storey, R.F., 2019. Very-low-dose twice-daily aspirin maintains platelet inhibition and improves haemostasis during dual-antiplatelet therapy for acute coronary syndrome. Platelets 30, 148–157. https://doi.org/10.1080/09537104.2019.1572880

Pedersen, M.T., Nørregaard, L.B., Jensen, T.D., Frederiksen, A.S., Ottesen, L., Bangsbo, J., 2022. The effect of 5 years of team sport on elderly males' health and social capital-An interdisciplinary follow-up study. Health Sci Rep 5, e760. https://doi.org/10.1002/hsr2.760

Pedersen, M.T., Vorup, J., Bangsbo, J., 2018. Effect of a 26-month floorball training on male elderly's cardiovascular fitness, glucose control, body composition, and functional capacity. J Sport Health Sci 7, 149–158. https://doi.org/10.1016/j.jshs.2017.12.002

Periayah, M.H., Halim, A.S., Mat Saad, A.Z., 2017. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. Int J Hematol Oncol Stem Cell Res 11, 319–327.

Perry, M.-C., Dufour, C.R., Tam, I.S., B'chir, W., Giguère, V., 2014. Estrogen-related receptor-α coordinates transcriptional programs essential for exercise tolerance and muscle fitness. Mol Endocrinol 28, 2060–2071. https://doi.org/10.1210/me.2014-1281

Pfitscher, A., Reiter, E., Jungbauer, A., 2008. Receptor binding and transactivation activities of red clover isoflavones and their metabolites. J Steroid Biochem Mol Biol 112, 87–94. https://doi.org/10.1016/j.jsbmb.2008.08.007

Phillips, D.R., 1985. Receptors for Platelet Agonists, in: George, J.N., Nurden, A.T., Phillips, D.R. (Eds.), Platelet Membrane Glycoproteins. Springer US, Boston, MA, pp. 145–169. https://doi.org/10.1007/978-1-4684-4880-1_7

Pierce, G.L., Eskurza, I., Walker, A.E., Fay, T.N., Seals, D.R., 2011. Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults. Clin Sci (Lond) 120, 13–23. https://doi.org/10.1042/CS20100174

Pignatelli, P., De Biase, L., Lenti, L., Tocci, G., Brunelli, A., Cangemi, R., Riondino, S., Grego, S., Volpe, M., Violi, F., 2005. Tumor necrosis factor-α as trigger of platelet activation in patients with heart failure. Blood 106, 1992–1994. https://doi.org/10.1182/blood-2005-03-1247

Pilote, L., Raparelli, V., 2018. Participation of Women in Clinical Trials: Not Yet Time to Rest on Our Laurels∗. Journal of the American College of Cardiology 71, 1970–1972. https://doi.org/10.1016/j.jacc.2018.02.069

Pilšáková, L., Riečanský, I., Jagla, F., 2010. The physiological actions of isoflavone phytoestrogens. Physiol Res 59, 651–664. https://doi.org/10.33549/physiolres.931902

Polo-Kantola, P., Erkkola, R., Irjala, K., Pullinen, S., Virtanen, I., Polo, O., 1999. Effect of short-term transdermal estrogen replacement therapy on sleep: a randomized, double-blind crossover trial in postmenopausal women. Fertility and Sterility 71, 873–880. https://doi.org/10.1016/S0015-0282(99)00062-X

Pomeroy, J.E., Helfer, A., Bursac, N., 2020. Biomaterializing the Promise of Cardiac Tissue Engineering. Biotechnol Adv 42, 107353. https://doi.org/10.1016/j.biotechadv.2019.02.009

Prior, J.C., 2020. Women's reproductive system as balanced estradiol and progesterone actions—A revolutionary, paradigm-shifting concept in women's health. Drug Discovery Today: Disease Models, Special section on Skin disease and Women's health 32, 31–40. https://doi.org/10.1016/j.ddmod.2020.11.005

Prossnitz, E.R., Barton, M., 2011. The G-protein-coupled estrogen receptor GPER in health and disease. Nat Rev Endocrinol 7, 715–726. https://doi.org/10.1038/nrendo.2011.122

Puca, A.A., Carrizzo, A., Villa, F., Ferrario, A., Casaburo, M., Maciąg, A., Vecchione, C., 2013. Vascular ageing: the role of oxidative stress. Int J Biochem Cell Biol 45, 556–559. https://doi.org/10.1016/j.biocel.2012.12.024

Rademaker, M., 2001. Do women have more adverse drug reactions? Am J Clin Dermatol 2, 349–351. https://doi.org/10.2165/00128071-200102060-00001

Rahman, M.M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., Almikhlafi, M.A., Alghamdi, S.Q., Alruwaili, A.S., Hossain, M.S., Ahmed, M., Das, R., Emran, T.B., Uddin, M.S., 2022. Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules 27, 233. https://doi.org/10.3390/molecules27010233

Randolph, J.F., Zheng, H., Sowers, M.R., Crandall, C., Crawford, S., Gold, E.B., Vuga, M., 2011. Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. J Clin Endocrinol Metab 96, 746–754. https://doi.org/10.1210/jc.2010-1746

Reed, B.G., Carr, B.R., 2018. The Normal Menstrual Cycle and the Control of Ovulation. MDText.com, Inc., Massachusetts.

Reuter, I., 2012. Aging, Physical Activity, and Disease Prevention 2012. J Aging Res 2012, 373294. https://doi.org/10.1155/2012/373294

Ribeiro, I. c., de Lucena, E. g. p., Oliveira, A. s., Fernandes, P. t., 2022. Physical exercise in menopause: a review of physiological changes. Comparative Exercise Physiology 18, 275–279. https://doi.org/10.3920/CEP220009

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Adams, R.J., Berry, J.D., Brown, T.M., Carnethon, M.R., Dai, S., de Simone, G., Ford, E.S., Fox, C.S., Fullerton, H.J., Gillespie, C., Greenlund, K.J., Hailpern, S.M., Heit, J.A., Ho, P.M., Howard, V.J., Kissela, B.M., Kittner, S.J., Lackland, D.T., Lichtman, J.H., Lisabeth, L.D., Makuc, D.M., Marcus, G.M., Marelli, A., Matchar, D.B., McDermott, M.M., Meigs, J.B., Moy, C.S., Mozaffarian, D., Mussolino, M.E., Nichol, G., Paynter, N.P., Rosamond, W.D., Sorlie, P.D., Stafford, R.S., Turan, T.N., Turner, M.B., Wong, N.D., Wylie-Rosett, J., 2011. Heart Disease and Stroke Statistics—2011 Update. Circulation 123, e18–e209. https://doi.org/10.1161/CIR.0b013e3182009701

Rosano, G.M.C., Vitale, C., Fini, M., 2009. Cardiovascular aspects of menopausal hormone replacement therapy. Climacteric 12, 41–46. https://doi.org/10.1080/13697130903012306

Rosenberg, R.D., Rosenberg, J.S., 1984. Natural anticoagulant mechanisms. J Clin Invest 74, 1–6.

Ross, R., 1999. Atherosclerosis--an inflammatory disease. N Engl J Med 340, 115–126. https://doi.org/10.1056/NEJM199901143400207

Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A.A., Howard, B.V., Johnson, K.C., Kotchen, J.M., Ockene, J., Writing Group for the Women's Health Initiative Investigators, 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 288, 321–333. https://doi.org/10.1001/jama.288.3.321

Rossouw, J.E., Prentice, R.L., Manson, J.E., Wu, L., Barad, D., Barnabei, V.M., Ko, M., LaCroix, A.Z., Margolis, K.L., Stefanick, M.L., 2007. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 297, 1465–1477. https://doi.org/10.1001/jama.297.13.1465

Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noël C., Beaton, A.Z., Benjamin, E.J., Benziger, C.P., Bonny, Aimé, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J., Catapano, A.L., Chugh, S.S., Cooper, L.T., Coresh, J., Criqui, M., DeCleene, N., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Solà, J., Fowkes, G., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N., Koroshetz, W., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Temesgen, A.M., Mokdad, A., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Moraes de Oliveira, G., Otto, C., Owolabi, M., Pratt, M., Rajagopalan, S., Reitsma, M., Ribeiro, A.L.P., Rigotti, N., Rodgers, A., Sable, C., Shakil, S., Sliwa-Hahnle, K., Stark, B., Sundström, J., Timpel, P., Tleyjeh, I.M., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L., Murray, C., Fuster, V., Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noel C., Beaton, A., Benjamin, E.J., Benziger, C.P., Bonny, Aime, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J.R., Catapano, A.L., Chugh, S., Cooper, L.T., Coresh, J., Criqui, M.H., DeCleene, N.K., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Sola, J., Fowkes, F.G.R., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N.J., Koroshetz, W.J., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Misganaw, A.T., Mokdad, A.H., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Oliveira, G.M.M., Otto, C.M., Owolabi, M.O., Pratt, M., Rajagopalan, S., Reitsma, M.B., Ribeiro, A.L.P., Rigotti, N.A., Rodgers, A., Sable, C.A., Shakil, S.S., Sliwa, K., Stark, B.A., Sundström, J., Timpel, P., Tleyjeh, I.I., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L.J., Abbasi-Kangevari, M., Abdi, A., Abedi, A., Aboyans, V., Abrha, W.A., Abu-Gharbieh, E., Abushouk, A.I., Acharya, D., Adair, T., Adebayo, O.M., Ademi, Z., Advani, S.M., Afshari, K., Afshin, A., Agarwal, G., Agasthi, P., Ahmad, S., Ahmadi, S., Ahmed, M.B., Aji, B., Akalu, Y., Akande-Sholabi, W., Aklilu, A., Akunna, C.J., Alahdab, F., Al-Eyadhy, A., Alhabib, K.F., Alif, S.M., Alipour, V., Aljunid, S.M., Alla, F., Almasi-Hashiani, A., Almustanyir, S., Al-Raddadi, R.M., Amegah, A.K., Amini, S., Aminorroaya, A., Amu, H., Amugsi, D.A., Ancuceanu, R., Anderlini, D., Andrei, T., Andrei, C.L., Ansari-Moghaddam, A., Anteneh, Z.A., Antonazzo, I.C., Antony, B., Anwer, R., Appiah, L.T., Arabloo, J., Ärnlöv, J., Artanti, K.D., Ataro, Z., Ausloos, M., Avila-Burgos, L., Awan, A.T., Awoke, M.A., Ayele, H.T., Ayza, M.A., Azari, S., B, D.B., Baheiraei, N., Baig, A.A., Bakhtiari, A., Banach, M., Banik, P.C., Baptista, E.A., Barboza, M.A., Barua, L., Basu, S., Bedi, N., Béjot, Y., Bennett, D.A., Bensenor, I.M., Berman, A.E., Bezabih, Y.M., Bhagavathula, A.S., Bhaskar, S., Bhattacharyya, K., Bijani, A., Bikbov, B., Birhanu, M.M., Boloor, A., Brant, L.C., Brenner, H., Briko, N.I., Butt, Z.A., Caetano dos Santos, F.L., Cahill, L.E., Cahuana-Hurtado, L., Cámera, L.A., Campos-Nonato, I.R., Cantu-Brito, C., Car, J., Carrero, J.J., Carvalho, F., Castañeda-Orjuela, C.A., Catalá-López, F., Cerin, E., Charan, J., Chattu, V.K., Chen, S., Chin, K.L., Choi, J.-Y.J., Chu, D.-T., Chung, S.-C., Cirillo, M., Coffey, S., Conti, S., Costa, V.M., Cundiff, D.K., Dadras, O., Dagnew, B., Dai, X., Damasceno, A.A.M., Dandona, L., Dandona, R., Davletov, K., De la Cruz-Góngora, V., De la Hoz, F.P., De Neve, J.-W., Denova-Gutiérrez, E., Derbew Molla, M., Derseh, B.T.,

Desai, R., Deuschl, G., Dharmaratne, S.D., Dhimal, M., Dhungana, R.R., Dianatinasab, M., Diaz, D., Djalalinia, S., Dokova, K., Douiri, A., Duncan, B.B., Duraes, A.R., Eagan, A.W., Ebtehaj, S., Eftekhari, A., Eftekharzadeh, S., Ekholuenetale, M., El Nahas, N., Elgendy, I.Y., Elhadi, M., El-Jaafary, S.I., Esteghamati, S., Etisso, A.E., Eyawo, O., Fadhil, I., Faraon, E.J.A., Faris, P.S., Farwati, M., Farzadfar, F., Fernandes, E., Fernandez Prendes, C., Ferrara, P., Filip, I., Fischer, F., Flood, D., Fukumoto, T., Gad, M.M., Gaidhane, S., Ganji, M., Garg, J., Gebre, A.K., Gebregiorgis, B.G., Gebregzabiher, K.Z., Gebremeskel, G.G., Getacher, L., Obsa, A.G., Ghajar, A., Ghashghaee, A., Ghith, N., Giampaoli, S., Gilani, S.A., Gill, P.S., Gillum, R.F., Glushkova, E.V., Gnedovskaya, E.V., Golechha, M., Gonfa, K.B., Goudarzian, A.H., Goulart, A.C., Guadamuz, J.S., Guha, A., Guo, Y., Gupta, R., Hachinski, V., Hafezi-Nejad, N., Haile, T.G., Hamadeh, R.R., Hamidi, S., Hankey, G.J., Hargono, A., Hartono, R.K., Hashemian, M., Hashi, A., Hassan, S., Hassen, H.Y., Havmoeller, R.J., Hay, S.I., Hayat, K., Heidari, G., Herteliu, C., Holla, R., Hosseini, M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Househ, M., Huang, J., Humayun, A., Iavicoli, I., Ibeneme, C.U., Ibitoye, S.E., Ilesanmi, O.S., Ilic, I.M., Ilic, M.D., Iqbal, U., Irvani, S.S.N., Islam, S.M.S., Islam, R.M., Iso, H., Iwagami, M., Jain, V., Javaheri, T., Jayapal, S.K., Jayaram, S., Jayawardena, R., Jeemon, P., Jha, R.P., Jonas, J.B., Jonnagaddala, J., Joukar, F., Jozwiak, J.J., Jürisson, M., Kabir, A., Kahlon, T., Kalani, R., Kalhor, R., Kamath, A., Kamel, I., Kandel, H., Kandel, A., Karch, A., Kasa, A.S., Katoto, P.D.M.C., Kayode, G.A., Khader, Y.S., Khammarnia, M., Khan, M.S., Khan, M.N., Khan, M., Khan, E.A., Khatab, K., Kibria, G.M.A., Kim, Y.J., Kim, G.R., Kimokoti, R.W., Kisa, S., Kisa, A., Kivimäki, M., Kolte, D., Koolivand, A., Korshunov, V.A., Koulmane Laxminarayana, S.L., Koyanagi, A., Krishan, K., Krishnamoorthy, V., Kuate Defo, B., Kucuk Bicer, B., Kulkarni, V., Kumar, G.A., Kumar, N., Kurmi, O.P., Kusuma, D., Kwan, G.F., La Vecchia, C., Lacey, B., Lallukka, T., Lan, Q., Lasrado, S., Lassi, Z.S., Lauriola, P., Lawrence, W.R., Laxmaiah, A., LeGrand, K.E., Li, M.-C., Li, B., Li, S., Lim, S.S., Lim, L.-L., Lin, H., Lin, Z., Lin, R.-T., Liu, X., Lopez, A.D., Lorkowski, S., Lotufo, P.A., Lugo, A., M, N.K., Madotto, F., Mahmoudi, M., Majeed, A., Malekzadeh, R., Malik, A.A., Mamun, A.A., Manafi, N., Mansournia, M.A., Mantovani, L.G., Martini, S., Mathur, M.R., Mazzaglia, G., Mehata, S., Mehndiratta, M.M., Meier, T., Menezes, R.G., Meretoja, A., Mestrovic, T., Miazgowski, B., Miazgowski, T., Michalek, I.M., Miller, T.R., Mirrakhimov, E.M., Mirzaei, H., Moazen, B., Moghadaszadeh, M., Mohammad, Y., Mohammad, D.K., Mohammed, S., Mohammed, M.A., Mokhayeri, Y., Molokhia, M., Montasir, A.A., Moradi, G., Moradzadeh, R., Moraga, P., Morawska, L., Moreno Velásquez, I., Morze, J., Mubarik, S., Muruet, W., Musa, K.I., Nagarajan, A.J., Nalini, M., Nangia, V., Naqvi, A.A., Narasimha Swamy, S., Nascimento, B.R., Nayak, V.C., Nazari, J., Nazarzadeh, M., Negoi, R.I., Neupane Kandel, S., Nguyen, H.L.T., Nixon, M.R., Norrving, B., Noubiap, J.J., Nouthe, B.E., Nowak, C., Odukoya, O.O., Ogbo, F.A., Olagunju, A.T., Orru, H., Ortiz, A., Ostroff, S.M., Padubidri, J.R., Palladino, R., Pana, A., Panda-Jonas, S., Parekh, U., Park, E.-C., Parvizi, M., Pashazadeh Kan, F., Patel, U.K., Pathak, M., Paudel, R., Pepito, V.C.F., Perianayagam, A., Perico, N., Pham, H.Q., Pilgrim, T., Piradov, M.A.,

Pishgar, F., Podder, V., Polibin, R.V., Pourshams, A., Pribadi, D.R.A., Rabiee, N., Rabiee, M., Radfar, A., Rafiei, A., Rahim, F., Rahimi-Movaghar, V., Ur Rahman, M.H., Rahman, M.A., Rahmani, A.M., Rakovac, I., Ram, P., Ramalingam, S., Rana, J., Ranasinghe, P., Rao, S.J., Rathi, P., Rawal, L., Rawasia, W.F., Rawassizadeh, R., Remuzzi, G., Renzaho, A.M.N., Rezapour, A., Riahi, S.M., Roberts-Thomson, R.L., Roever, L., Rohloff, P., Romoli, M., Roshandel, G., Rwegerera, G.M., Saadatagah, S., Saber-Ayad, M.M., Sabour, S., Sacco, S., Sadeghi, M., Saeedi Moghaddam, S., Safari, S., Sahebkar, A., Salehi, S., Salimzadeh, H., Samaei, M., Samy, A.M., Santos, I.S., Santric-Milicevic, M.M., Sarrafzadegan, N., Sarveazad, A., Sathish, T., Sawhney, M., Saylan, M., Schmidt, M.I., Schutte, A.E., Senthilkumaran, S., Sepanlou, S.G., Sha, F., Shahabi, S., Shahid, I., Shaikh, M.A., Shamali, M., Shamsizadeh, M., Shawon, M.S.R., Sheikh, A., Shigematsu, M., Shin, M.-J., Shin, J.I., Shiri, R., Shiue, I., Shuval, K., Siabani, S., Siddiqi, T.J., Silva, D.A.S., Singh, J.A., Mtech, A.S., Skryabin, V.Y., Skryabina, A.A., Soheili, A., Spurlock, E.E., Stockfelt, L., Stortecky, S., Stranges, S., Suliankatchi Abdulkader, R., Tadbiri, H., Tadesse, E.G., Tadesse, D.B., Tajdini, M., Tariqujjaman, M., Teklehaimanot, B.F., Temsah, M.-H., Tesema, A.K., Thakur, B., Thankappan, K.R., Thapar, R., Thrift, A.G., Timalsina, B., Tonelli, M., Touvier, M., Tovani-Palone, M.R., Tripathi, A., Tripathy, J.P., Truelsen, T.C., Tsegay, G.M., Tsegaye, G.W., Tsilimparis, N., Tusa, B.S., Tyrovolas, S., Umapathi, K.K., Unim, B., Unnikrishnan, B., Usman, M.S., Vaduganathan, M., Valdez, P.R., Vasankari, T.J., Velazquez, D.Z., Venketasubramanian, N., Vu, G.T., Vujcic, I.S., Waheed, Y., Wang, Y., Wang, F., Wei, J., Weintraub, R.G., Weldemariam, A.H., Westerman, R., Winkler, A.S., Wiysonge, C.S., Wolfe, C.D.A., Wubishet, B.L., Xu, G., Yadollahpour, A., Yamagishi, K., Yan, L.L., Yandrapalli, S., Yano, Y., Yatsuya, H., Yeheyis, T.Y., Yeshaw, Y., Yilgwan, C.S., Yonemoto, N., Yu, C., Yusefzadeh, H., Zachariah, G., Zaman, S.B., Zaman, M.S., Zamanian, M., Zand, R., Zandifar, A., Zarghi, A., Zastrozhin, M.S., Zastrozhina, A., Zhang, Z.-J., Zhang, Y., Zhang, W., Zhong, C., Zou, Z., Zuniga, Y.M.H., Murray, C.J.L., Fuster, V., 2020. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology 76, 2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010

Sabra, A., Lawrence, M.J., Aubrey, R., Obaid, D.R., Chase, A.J., Smith, D., Thomas, P., Storton, S., Davies, G., Hawkins, K., Morris, K., Evans, P.A., 2017. Characterisation of clot microstructure properties in stable coronary artery disease | Open Heart [WWW Document]. URL https://openheart.bmj.com/content/4/2/e000562.abstract (accessed 9.18.22).

Sadler, J.E., 1998. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem 67, 395–424. https://doi.org/10.1146/annurev.biochem.67.1.395

Samuels, C., 2009. Sleep, recovery, and performance: the new frontier in high-performance athletics. Phys Med Rehabil Clin N Am 20, 149–159, ix. https://doi.org/10.1016/j.pmr.2008.10.009

Santoro, N., Epperson, C.N., Mathews, S.B., 2015. Menopausal Symptoms and Their Management. Endocrinol Metab Clin North Am 44, 497–515. https://doi.org/10.1016/j.ecl.2015.05.001

Santoro, N., Randolph, J.F., 2011. Reproductive Hormones and the Menopause Transition. Obstet Gynecol Clin North Am 38, 455–466. https://doi.org/10.1016/j.ogc.2011.05.004

Santos-Parker, J.R., Strahler, T.R., Vorwald, V.M., Pierce, G.L., Seals, D.R., 2017. Habitual aerobic exercise does not protect against micro- or macrovascular endothelial dysfunction in healthy estrogen-deficient postmenopausal women. J Appl Physiol (1985) 122, 11–19. https://doi.org/10.1152/japplphysiol.00732.2016

Sathyapalan, T., Aye, M., Rigby, A.S., Thatcher, N.J., Dargham, S.R., Kilpatrick, E.S., Atkin, S.L., 2018. Soy isoflavones improve cardiovascular disease risk markers in women during the early menopause. Nutr Metab Cardiovasc Dis 28, 691–697. https://doi.org/10.1016/j.numecd.2018.03.007

Savi, P., Nurden, P., Nurden, A.T., Levy-Toledano, S., Herbert, J.M., 1998. Clopidogrel: a review of its mechanism of action. Platelets 9, 251–255. https://doi.org/10.1080/09537109876799

Scharhag-Rosenberger, F., Meyer, T., Gässler, N., Faude, O., Kindermann, W., 2010. Exercise at given percentages of VO2max: heterogeneous metabolic responses between individuals. J Sci Med Sport 13, 74–79. https://doi.org/10.1016/j.jsams.2008.12.626

Schmaier, A.H., 2008. The elusive physiologic role of Factor XII. J Clin Invest 118, 3006–3009. https://doi.org/10.1172/JCI36617

Schmidt, J.F., Hansen, P.R., Andersen, T.R., Andersen, L.J., Hornstrup, T., Krustrup, P., Bangsbo, J., 2014. Cardiovascular adaptations to 4 and 12 months of football or strength training in 65- to 75-year-old untrained men. Scand J Med Sci Sports 24 Suppl 1, 86–97. https://doi.org/10.1111/sms.12217

Schreiber, S.N., Emter, R., Hock, M.B., Knutti, D., Cardenas, J., Podvinec, M., Oakeley, E.J., Kralli, A., 2004. The estrogen-related receptor α (ERRα) functions in PPARγ coactivator 1α (PGC-1α)-induced mitochondrial biogenesis. Proceedings of the National Academy of Sciences 101, 6472–6477. https://doi.org/10.1073/pnas.0308686101

Schuff-Werner, P., Steiner, M., Fenger, S., Gross, H.-J., Bierlich, A., Dreissiger, K., Mannuß, S., Siegert, G., Bachem, M., Kohlschein, P., 2013. Effective estimation of correct platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. Br J Haematol 162, 684–692. https://doi.org/10.1111/bjh.12443

Seals, D.R., Jablonski, K.L., Donato, A.J., 2011. Aging and vascular endothelial function in humans. Clinical Science 120, 357–375. https://doi.org/10.1042/CS20100476

Seidelin, K., Nyberg, M., Piil, P., Jørgensen, N.R., Hellsten, Y., Bangsbo, J., 2017. Adaptations with Intermittent Exercise Training in Post- and Premenopausal Women. Medicine & Science in Sports & Exercise 49, 96–105. https://doi.org/10.1249/MSS.0000000000001071

Shibata, S., Fujimoto, N., Hastings, J.L., Carrick-Ranson, G., Bhella, P.S., Hearon Jr., C.M., Levine, B.D., 2018. The effect of lifelong exercise frequency on arterial stiffness. The Journal of Physiology 596, 2783–2795. https://doi.org/10.1113/JP275301

Simoncini, T., Garibaldi, S., Fu, X.-D., Pisaneschi, S., Begliuomini, S., Baldacci, C., Lenzi, E., Goglia, L., Giretti, M.S., Genazzani, A.R., 2008. Effects of phytoestrogens derived from red clover on atherogenic adhesion molecules in human endothelial cells. Menopause 15, 542–550. https://doi.org/10.1097/gme.0b013e318156f9d6

Smith, E.S., McKay, A.K.A., Kuikman, M., Ackerman, K.E., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., Burke, L.M., 2022. Auditing the Representation of Female Versus Male Athletes in Sports Science and Sports Medicine Research: Evidence-Based Performance Supplements. Nutrients 14, 953. https://doi.org/10.3390/nu14050953

Smith, L.L., 2000. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? Med Sci Sports Exerc 32, 317–331. https://doi.org/10.1097/00005768-200002000-00011

Sowers, M.R., Eyvazzadeh, A.D., McConnell, D., Yosef, M., Jannausch, M.L., Zhang, D., Harlow, S., Randolph, J.F., 2008. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab 93, 3478–3483. https://doi.org/10.1210/jc.2008-0567

Spalding, A., Vaitkevicius, H., Dill, S., MacKenzie, S., Schmaier, A., Lockette, W., 1998. Mechanism of epinephrine-induced platelet aggregation. Hypertension 31, 603–607. https://doi.org/10.1161/01.hyp.31.2.603

Spina, R.J., Ogawa, T., Kohrt, W.M., Martin, W.H., Holloszy, J.O., Ehsani, A.A., 1993. Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol (1985) 75, 849–855. https://doi.org/10.1152/jappl.1993.75.2.849

Squadrito, F., Altavilla, D., Morabito, N., Crisafulli, A., D'Anna, R., Corrado, F., Ruggeri, P., Campo, G.M., Calapai, G., Caputi, A.P., Squadrito, G., 2002. The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. Atherosclerosis 163, 339–347. https://doi.org/10.1016/S0021-9150(02)00013-8

Staessen, J.A., Ginocchio, G., Thijs, L., Fagard, R., 1997. Conventional and ambulatory blood pressure and menopause in a prospective population study. J Hum Hypertens 11, 507–514. https://doi.org/10.1038/sj.jhh.1000476

Stokes, K.Y., Granger, D.N., 2012. Platelets: a critical link between inflammation and microvascular dysfunction. J Physiol 590, 1023–1034. https://doi.org/10.1113/jphysiol.2011.225417

Su, H.I., Freeman, E.W., 2009. Hormone changes associated with the menopausal transition. Minerva Ginecol 61, 483–489.

Thomas, P., Pang, Y., 2013. Protective actions of progesterone in the cardiovascular system: potential role of membrane progesterone receptors (mPRs) in mediating rapid effects. Steroids 78, 583–588. https://doi.org/10.1016/j.steroids.2013.01.003

Thompson, W.R., Sallis, R., Joy, E., Jaworski, C.A., Stuhr, R.M., Trilk, J.L., 2020. Exercise Is Medicine. American Journal of Lifestyle Medicine 14, 511–523. https://doi.org/10.1177/1559827620912192

Thorp, J.A., Walsh, S.W., Brath, P.C., 1988. Low-dose aspirin inhibits thromboxane, but not prostacyclin, production by human placental arteries. Am J Obstet Gynecol 159, 1381–1384. https://doi.org/10.1016/0002-9378(88)90560-1

Thorup, A.C., Lambert, M.N., Kahr, H.S., Bjerre, M., Jeppesen, P.B., 2015. Intake of Novel Red Clover Supplementation for 12 Weeks Improves Bone Status in Healthy Menopausal Women. Evid Based Complement Alternat Med 2015, 689138. https://doi.org/10.1155/2015/689138

Timmons, J.A., Knudsen, S., Rankinen, T., Koch, L.G., Sarzynski, M., Jensen, T., Keller, P., Scheele, C., Vollaard, N.B.J., Nielsen, S., Akerström, T., MacDougald, O.A., Jansson, E., Greenhaff, P.L., Tarnopolsky, M.A., van Loon, L.J.C., Pedersen, B.K., Sundberg, C.J., Wahlestedt, C., Britton, S.L., Bouchard, C., 2010. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. J Appl Physiol (1985) 108, 1487–1496. https://doi.org/10.1152/japplphysiol.01295.2009

Ting, Y., Jiang, Y., Ho, C.-T., Huang, Q., 2014. Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals. Journal of Functional Foods 7, 112–128. https://doi.org/10.1016/j.jff.2013.12.010

Tofas, T., Draganidis, D., Deli, C.K., Georgakouli, K., Fatouros, I.G., Jamurtas, A.Z., 2020. Exercise-Induced Regulation of Redox Status in Cardiovascular Diseases: The Role of Exercise Training and Detraining. Antioxidants 9, 13. https://doi.org/10.3390/antiox9010013

Trifan, G., Gorelick, P.B., Testai, F.D., 2021. Efficacy and Safety of Using Dual Versus Monotherapy Antiplatelet Agents in Secondary Stroke Prevention: Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. Circulation 143, 2441–2453. https://doi.org/10.1161/CIRCULATIONAHA.121.053782

Tripathi, M., Yen, P.M., Singh, B.K., 2020. Estrogen-Related Receptor Alpha: An Under-Appreciated Potential Target for the Treatment of Metabolic Diseases. Int J Mol Sci 21, 1645. https://doi.org/10.3390/ijms21051645

Troy, G.C., 1988. An Overview of Hemostasis. Veterinary Clinics of North America: Small Animal Practice 18, 5–20. https://doi.org/10.1016/S0195-5616(88)50003-7

van Beek, J.H.G.M., Kirkwood, T.B.L., Bassingthwaighte, J.B., 2016. Understanding the physiology of the ageing individual: computational modelling of changes in metabolism and endurance. Interface Focus 6, 20150079. https://doi.org/10.1098/rsfs.2015.0079

Verdoia, M., Suryapranata, H., Damen, S., Camaro, C., Benit, E., Barbieri, L., Rasoul, S., Liew, H.B., Polad, J., Ahmad, W. a. W., Zambahari, R., Lalmand, J., van der Schaaf, R.J., Koh, T.H., Timmermans, P., Dilling-Boer, D., Veenstra, L.F., Van't Hof, A.W.J., Lee, S.W.L., Roolvink, V., Ligtenberg, E., Postma, S., Kolkman, E.J.J., Brouwer, M.A., Kedhi, E., De Luca, G., 2021. Gender differences with short-term vs 12 months dual antiplatelet therapy in patients with acute coronary syndrome treated with the COMBO dual therapy stent: 2-years follow-up results of the

REDUCE trial. J Thromb Thrombolysis 52, 797–807. https://doi.org/10.1007/s11239-021-02439-x
Viggiani, M.T., Polimeno, L., Di Leo, A., Barone, M., 2019. Phytoestrogens: Dietary Intake, Bioavailability, and Protective Mechanisms against Colorectal Neoproliferative Lesions. Nutrients 11, 1709. https://doi.org/10.3390/nu11081709
Villena, J.A., Kralli, A., 2008. ERRα: a metabolic function for the oldest orphan. Trends Endocrinol Metab 19, 269–276. https://doi.org/10.1016/j.tem.2008.07.005
Vitale, D.C., Piazza, C., Melilli, B., Drago, F., Salomone, S., 2013. Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet 38, 15–25. https://doi.org/10.1007/s13318-012-0112-y
Vorup, J., Pedersen, M.T., Brahe, L.K., Melcher, P.S., Alstrøm, J.M., Bangsbo, J., 2017. Effect of small-sided team sport training and protein intake on muscle mass, physical function and markers of health in older untrained adults: A randomized trial. PLoS One 12, e0186202. https://doi.org/10.1371/journal.pone.0186202
Wagner, P.D., 2011. The critical role of VEGF in skeletal muscle angiogenesis and blood flow. Biochem Soc Trans 39, 1556–1559. https://doi.org/10.1042/BST20110646
Wallace, R.B., Sherman, B.M., Bean, J.A., Treloar, A.E., Schlabaugh, L., 1979. Probability of menopause with increasing duration of amenorrhea in middle-aged women. Am J Obstet Gynecol 135, 1021–1024. https://doi.org/10.1016/0002-9378(79)90729-4
Wang, G.-R., Zhu, Y., Halushka, P.V., Lincoln, T.M., Mendelsohn, M.E., 1998. Mechanism of platelet inhibition by nitric oxide: In vivo phosphorylation of thromboxane receptor by cyclic GMP-dependent protein kinase. Proc Natl Acad Sci U S A 95, 4888–4893.
Warlo, E.M.K., Arnesen, H., Seljeflot, I., 2019. A brief review on resistance to P2Y12 receptor antagonism in coronary artery disease. Thrombosis Journal 17, 11. https://doi.org/10.1186/s12959-019-0197-5
Warner, T.D., Armstrong, P.C., Chan, M.V., Knowles, R.B., 2016. The importance of endothelium-derived mediators to the efficacy of dual anti-platelet therapy. Expert Rev Hematol 9, 223–225. https://doi.org/10.1586/17474086.2016.1140035
Warner, T.D., Nylander, S., Whatling, C., 2011. Anti-platelet therapy: cyclo-oxygenase inhibition and the use of aspirin with particular regard to dual anti-platelet therapy. Br J Clin Pharmacol 72, 619–633. https://doi.org/10.1111/j.1365-2125.2011.03943.x
Weisel, J.W., Litvinov, R.I., 2017. Fibrin Formation, Structure and Properties. Subcell Biochem 82, 405–456. https://doi.org/10.1007/978-3-319-49674-0_13
Welt, C.K., McNicholl, D.J., Taylor, A.E., Hall, J.E., 1999. Female reproductive aging is marked by decreased secretion of dimeric inhibin. J Clin Endocrinol Metab 84, 105–111. https://doi.org/10.1210/jcem.84.1.5381
Wendelboe, A.M., Raskob, G.E., 2016. Global Burden of Thrombosis. Circulation Research 118, 1340–1347. https://doi.org/10.1161/CIRCRESAHA.115.306841

Westby, C.M., Weil, B.R., Greiner, J.J., Stauffer, B.L., Desouza, C.A., 2011. Endothelin-1 vasoconstriction and the age-related decline in endothelium-dependent vasodilatation in men. Clinical Science 120, 485–491. https://doi.org/10.1042/CS20100475

White, J.G., 1998. Use of the electron microscope for diagnosis of platelet disorders. Semin Thromb Hemost 24, 163–168. https://doi.org/10.1055/s-2007-995836

Whorton, A.R., Willis, C.E., Kent, R.S., Young, S.L., 1984. The role of calcium in the regulation of prostacyclin synthesis by porcine aortic endothelial cells. Lipids 19, 17–24. https://doi.org/10.1007/BF02534603

Wickham, K.A., Spriet, L.L., 2023. Food for Thought: Physiological Considerations for Nutritional Ergogenic Efficacy. Scandinavian Journal of Medicine & Science in Sports n/a. https://doi.org/10.1111/sms.14307

Wiik, A., Hellsten, Y., Berthelson, P., Lundholm, L., Fischer, H., Jansson, E., 2009. Activation of estrogen response elements is mediated both via estrogen and muscle contractions in rat skeletal muscle myotubes. Am J Physiol Cell Physiol 296, C215-220. https://doi.org/10.1152/ajpcell.00148.2008

Wilkerson, W.R., Sane, D.C., 2002. Aging and thrombosis. Semin Thromb Hemost 28, 555–568. https://doi.org/10.1055/s-2002-36700

Willhite, L.A., O'Connell, M.B., 2001. Urogenital Atrophy: Prevention and Treatment. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 21, 464–480. https://doi.org/10.1592/phco.21.5.464.34486

World Health Organization, 2022. Cardiovascular diseases [WWW Document]. URL https://www.who.int/health-topics/cardiovascular-diseases (accessed 10.12.22).

Woulfe, D., Yang, J., Brass, L., 2001. ADP and platelets: the end of the beginning. J Clin Invest 107, 1503–1505.

Xia, H., Dufour, C.R., Giguère, V., 2019. ERRα as a Bridge Between Transcription and Function: Role in Liver Metabolism and Disease. Frontiers in Endocrinology 10.

Xu, X., Wang, B., Ren, C., Hu, J., Greenberg, D.A., Chen, T., Xie, L., Jin, K., 2017. Age-related Impairment of Vascular Structure and Functions. Aging Dis 8, 590–610. https://doi.org/10.14336/AD.2017.0430

Xu, Y., Shen, Y., Chen, D., Zhao, P., Jiang, J., 2021. Efficacy and Safety of Dual Antiplatelet Therapy in Patients Undergoing Coronary Stent Implantation: A Systematic Review and Network Meta-Analysis. Journal of Interventional Cardiology 2021, e9934535. https://doi.org/10.1155/2021/9934535

Yaffe, K., Sawaya, G., Lieberburg, I., Grady, D., 1998. Estrogen Therapy in Postmenopausal WomenEffects on Cognitive Function and Dementia. JAMA 279, 688–695. https://doi.org/10.1001/jama.279.9.688

Yousefzadeh, M., Henpita, C., Vyas, R., Soto-Palma, C., Robbins, P., Niedernhofer, L., 2021. DNA damage-how and why we age? Elife 10, e62852. https://doi.org/10.7554/eLife.62852

Yuan, S.Y., Rigor, R.R., 2010. Structure and Function of Exchange Microvessels, Regulation of Endothelial Barrier Function. Morgan & Claypool Life Sciences.

Yun, S.-H., Sim, E.-H., Goh, R.-Y., Park, J.-I., Han, J.-Y., 2016. Platelet Activation: The Mechanisms and Potential Biomarkers. BioMed Research International 2016, e9060143. https://doi.org/10.1155/2016/9060143

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J., Lisheng, L., INTERHEART Study Investigators, 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 364, 937–952. https://doi.org/10.1016/S0140-6736(04)17018-9

Zhong, P.-Y., Shang, Y.-S., Bai, N., Ma, Y., Niu, Y., Wang, Z.-L., 2021. Efficacy and Safety of Very Short-Term Dual Antiplatelet Therapy After Drug-Eluting Stents Implantation for Acute Coronary Syndrome: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Front Cardiovasc Med 8, 660360. https://doi.org/10.3389/fcvm.2021.660360

6.7 Research Program Progression

In *Chapter 6*, we validated the *exercise timing hypothesis* for the first time by showing that the vascular adaptations to an exercise training program are divergent between recently postmenopausal and late postmenopausal women. Specifically, recently postmenopausal women exhibit beneficial vascular adaptations to as little as 8 weeks of high-intensity exercise training, however this response is not observed in late postmenopausal women. Accordingly, taking into account the findings from *Chapter 5 and 6*, we only recruited recently postmenopausal women to study the effects of short-term phytoestrogen supplementation on markers of vascular health in *Chapter 7*. We believed this would afford us the greatest opportunity to observe a beneficial effect of the short-term supplementation protocol.

CHAPTER 7

Study III – Short-term supplementation with fermented red clover extract reduces vascular inflammation in early post-menopausal women

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7.1 Abstract

The decline in oestrogen at menopause poses a critical challenge to cardiovascular and metabolic health. Recently, a growing interest in the role of phytoestrogens, with a particular focus on isoflavones, has emerged as they can bind to estrogen receptors and may mimic the roles of endogenous oestrogen. Fermented red clover extract contains isoflavones with superior bioavailability compared to non-fermented isoflavones, however little is known regarding the impact of isoflavones on cardiovascular and metabolic health. We assessed markers of vascular health in plasma and skeletal muscle samples obtained from healthy but sedentary early post-menopausal women (n = 10; 54 ± 4 yrs) following two weeks of twice daily treatment with placebo or fermented red clover extract (60 mg isoflavones per day). The two interventions were administered using a randomized, double-blind, crossover design with a two-week washout period. Plasma

samples were utilized for assessment of markers of vascular inflammation. There was a statistically significant reduction (~5.4%) in vascular cell adhesion molecule 1 (VCAM-1) following two weeks of fermented red clover extract supplementation compared to placebo ($p = 0.03$). In contrast, there was no effect of fermented red clover extract supplementation compared to placebo on skeletal muscle oestrogen receptor content and enzymes related to vascular function, and angiogenesis. Supplementation with fermented red clover extract reduces vascular inflammation in early post-menopausal women and future studies should address the long-term impact of daily supplementation with fermented red clover extract after menopause.

7.2 Introduction

Existing evidence clearly highlights that the loss of oestrogen associated with menopause poses a critical challenge to cardiovascular and metabolic health (Mendelsohn and Karas, 1999; Mikkola et al., 2013). Notably, compared to age-matched men, post-menopausal women have a similar, if not greater, risk of developing cardiovascular and metabolic disease (Lerner and Kannel, 1986; Messerli et al., 1987; Taddei et al., 1996; Barrett-Connor, 1997). In pre-menopausal women, oestrogen plays a cardioprotective role through the activation of several signalling cascades, which lead to the promotion of vasodilation and angiogenesis as well as the reduction in oxidative stress and fibrosis (Iorga et al., 2017). Additionally, the biochemical structure of oestrogen possesses direct anti-inflammatory characteristics, where the hydroxyl group of its aromatic ring can quench superoxide ions (Dubey and Jackson, 2001). Accordingly, the withdrawal of oestrogen production associated with menopause has been shown to increase oxidative

stress and subsequently induce negative effects on cardiovascular health including increased sympathetic tone, endothelial dysfunction, vascular inflammation, and increased blood pressure (Maas and Franke, 2009; Novella et al., 2012). Moreover, endothelial dysfunction and subsequent alterations in vascular tone and blood flow have significant implications for peripheral tissue metabolism, whereby skeletal muscle angiogenesis can become impaired (Hodges et al., 2018; Olsen et al., 2020).

Hormone replacement therapy (HRT) has been a leading therapeutic intervention for the maintenance of cardiovascular and metabolic health in post-menopausal women (Grady et al., 1992). However, there is mounting concern regarding the efficacy and safety of this treatment (Naftolin et al., 2004). Specifically, it appears that HRT may not be an effective strategy for mitigating cardiovascular disease development if started late into menopause (Miller et al., 2009; Hodis et al., 2016). Consequently, there is an urgent call for novel, safe, and efficacious therapeutic interventions that can promote cardiovascular and metabolic health in post-menopausal women. Recently, a growing interest in the role of phytoestrogens, with a particular focus on isoflavones, has emerged. Isoflavones are predominantly derived from legumes and can bind to both oestrogen receptor alpha (ERα) and oestrogen receptor beta (ERβ), thereby potentially mimicking the roles of endogenous oestrogen (Pfitscher et al., 2008; Pilsakova et al., 2010). Interestingly, although 17β-estradiol binds with equal affinity to ERα and ERβ (Couse et al., 1997), phytoestrogens appear to have a higher affinity for ERβ (Pfitscher et al., 2008). Epidemiological data suggests that women ingesting large amounts of phytoestrogens are less likely to develop cardiovascular disease as well as breast and uterine cancer (Horn-

Ross et al., 2003; Sathyapalan et al., 2018). Specifically, long-term (≥ 6 weeks) soy isoflavone supplementation has been linked to increased nitric oxide (NO) bioavailability (Squadrito et al., 2003) and prostacyclin (PGI_2) release (Garcia-Martinez et al., 2003) as well as reduced endothelin-1 levels (Squadrito et al., 2003) in post-menopausal women. Moreover, a growing body of evidence suggests that long-term soy isoflavone supplementation can improve markers on vascular inflammation in post-menopausal women (Colacurci et al., 2005; Hall et al., 2005; Lebon et al., 2014). Importantly, these findings translate to an improvement in vascular function (Li et al., 2010).

Red clover (trifolium pratense), a plant known for its high phytoestrogen content, is beginning to gain traction as a novel therapeutic intervention for post-menopausal women as it is rich in the isoflavones biochanin A and formonentin (Lemeziene et al., 2015). Though limited, previous research has shown that supplementation with red clover extract can confer positive effects on vascular function in post-menopausal women (Nestel et al., 1999; Howes et al., 2003). However, the findings regarding blood markers of cardiovascular health are currently unclear. Some evidence suggests improvements in antioxidant and vasorelaxant properties (Kim et al., 2020) as well as atherogenic adhesion molecules (Simoncini et al., 2008), while others demonstrate no effect of red clover extract on inflammatory markers (Thorup et al., 2015) or coagulation factors (Mainini et al., 2013). A key determinant of nutraceutical efficacy is the bioavailability of the supplement (Ting et al., 2014). In recent years, advances in food science and nutraceutical biochemistry have significantly improved the bioavailability, and therefore, the therapeutic potential of red clover isoflavones via the fermentation process, which

converts isoflavone glycosides to aglycones (Thorup et al., 2015). Therefore, fermented red clover extract may be a safe, easily accessible, and highly efficacious therapeutic intervention to promote cardiovascular and metabolic health as well as bone health (Thorup et al., 2015) and the maintenance of muscle mass and function (Oxfeldt et al., 2020) in post-menopausal women. However, to date, no study has evaluated the cardiovascular benefits in a cohort of post-menopausal women. Therefore, we aimed to determine whether two weeks of supplementation with fermented red clover extract could positively influence markers of vascular health in blood and skeletal muscle in early post-menopausal women utilizing a crossover design. We hypothesized that short-term supplementation with fermented red clover extract would significantly improve markers of vascular health in the blood and skeletal muscle of early post-menopausal women.

7.3 Methods

7.3.1 Ethical Approval

The human muscle biopsies and blood samples included in the present study originated from a published study conducted at the Department of Public Health, Aarhus University, Denmark (Oxfeldt et al., 2020). The study was in accordance with the Declaration of Helsinki and was approved by the Central Denmark Region Committees on Health Research Ethics (1-10-72-212-19) and registered at Clinical.trials.gov (ID: NCT04154206). All the participants provided written informed consent to participate after they were fully informed about the project as well as the associated risks and discomforts.

7.3.2 Participants

Ten healthy but sedentary, early post-menopausal women were recruited to participate in this study (age: 54 ± 4 yrs; height: 168 ± 6 cm; weight: 70 ± 8 kg) via posters displayed in the local area and on social media. Women were defined as early post-menopausal if they had not menstruated for at least 6 months, but it had been less than 5 years since their last menstrual bleeding (8 to 33 months since last menstruation). Participants were excluded from the study if they met any of the following criteria: body mass index (BMI) > 30, regularly participated in ≥ 3 hours of training per week (except for bike transport; < 70 km per week), use of hormone replacement therapy or isoflavone supplements, ovariectomy, cardiovascular and metabolic diseases, as well as injuries or use of medication affecting skeletal muscle.

7.3.3 Study Design

Using a double-blind, randomized, crossover design, participants completed two different 14-day intervention periods: fermented red clover extract and placebo. The intervention periods were separated by a two-week washout period (14 ± 0 days). On the final day (Day 14) of each experimental period, the participants arrived at the laboratory after an overnight fast. Resting blood samples were obtained from the antecubital vein, where 3.5 mL was collected in a lithium heparin-coated tube. A muscle biopsy was obtained from the middle of the m. vastus lateralis using a Bergström needle with suction under local anesthesia (Figure 7-1). All muscle samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

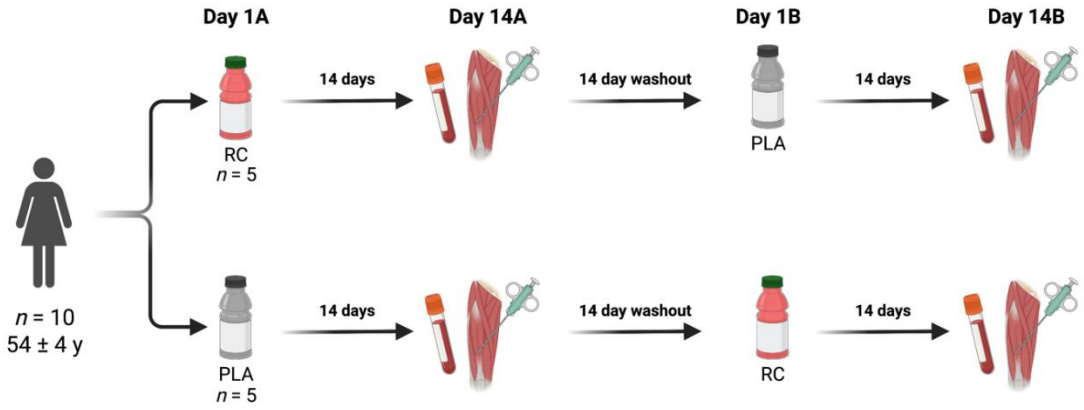

Figure 7-1: Study design overview. Created with BioRender.com.

7.3.4 Experimental Drinks

The fermented red clover extract is a commercially available product produced by Herrens Mark Aps (Nørre Aaby, Denmark), and it is comprised of juice from pressed red clover plants mixed with probiotic lactic acid bacteria to facilitate cold fermentation. These bacteria promote the conversion of isoflavone glucosides to aglycones, which improves the bioavailability of the fermented red clover extract. The research team added stevia and natural sugar-free raspberry flavouring to the fermented red clover extract to mask the flavour and appearance of the product. The placebo drink consisted of water with added food colouring as well as stevia and sugar-free raspberry flavouring to match the fermented red clover extract product. The two drinks were sealed and packaged in identical boxes labelled “A” or “B” to ensure adequate blinding of the participants and members of the research team.

The fermented red clover extract and placebo drinks were provided to the participants on the first day of each intervention period. Participants were instructed to consume a

daily dose of 120 mL, distributed as 60 mL in the morning and 60 mL in the evening. This dosing regime delivered a minimum of 60 mg isoflavones per day and has been shown to significantly elevate plasma isoflavone levels (Lambert et al., 2017b). At the end of the 14-day intervention period, the participants returned the empty containers to the research team as a measure of compliance, which was 100%.

7.3.5 Blood Analysis

Venous blood was centrifuged immediately (10 minutes at 1300 rpm and 5°C). Aliquoted plasma was stored at -80°C until later analysis. Plasma levels of inflammatory markers associated with vascular damage (vascular cell adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), serum amyloid A (SAA), and C-reactive protein (CRP)) were measured in accordance with the manufacturer's instructions via an electrochemiluminescent assay kit (V-PLEX Vascular Injury Panel 2, Meso Scale Diagnostics, USA). Plasma markers are presented for 9 subjects due to difficulties collecting blood from one participant.

7.3.6 Western Blotting

Approximately 20 to 50 mg of skeletal muscle was used to determine protein content of oestrogen and oestrogen-related receptors as well as microvascular enzymes and angiogenic proteins via western blot analysis as previously described (Hoier et al., 2012). Briefly, the muscle samples were freeze dried and dissected free of fat, blood, and connective tissue. The samples were then homogenized in a fresh batch of buffer (10% glycerol, 20 mM sodium pyrophosphate, 150 mM NaCl, 50 mM HEPES (pH 7.5), 1% NP-40, 20 mM β-glycerophosphate, 2 mM Na3VO4, 10 mM NaF, 2 mM PMSF, 1 mM EDTA

(pH 8.0), 1 mM EGTA (pH 8.0), 10 µg/mL aprotinin, 10 µg/mL leupeptin, 3 mM benzamidine) twice for 30 s each (Qiagen Tissuelyser II; Retsch, Haan, Germany). After rotation end-over-end for 1 hour at 4°C, the suspension was centrifuged, and the lysate was collected for analysis. The concentration of total proteins in the lysate was determined in triplicate via BCA protein assay (Pierce Biotechnology Inc., Rockwood, IL, USA). All samples were loaded in duplicates and samples from the same subject were always loaded on the same gel. TGX stain-free gels were used as loading controls, whereby equal protein loading was confirmed by total protein determination from the stain-free image. After gel electrophoresis, proteins were semi-dry transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon Transfer Membrane, Millipore, MA, USA), which was blocked before overnight incubation with primary antibodies for oestrogen receptor alpha (ERα; cs 8644; 1:1000), oestrogen receptor beta (ERβ; mab 7106; 1:500), oestrogen-related receptor alpha (ERRα; ab76228; 1:750), fetal liver kinase 1 (VEGFR2, Flk-1; sc 393163; 1:300), cluster of differentiation 31 (CD31; af 806; 1:250), superoxide dismutase 2 (SOD2; Millipore 06-984; 1:5000), endothelial nitric oxide synthase (eNOS; ab76198; 1:1000), and prostacyclin synthase (PGI_2S; ab23668; 1:300) (Figure 7-2). Thereafter, membranes were washed for 5 min in Tris-buffered saline Tween (TBST) before incubation with secondary horseradish peroxidase (HRP) conjugated antibody for 1 hour. Then, the membrane was washed three times for 5 min in TBST. Membrane staining was visualized by incubation with a chemiluminescent HRP substrate. ERα, ERβ, ERRα, CD31, SOD2, and PGI_2S were imaged using Luminata Forte (ECL; Merck Millipore, Darmstadt, Germany), and Flk-1 and eNOS were imaged using SuperSignal™

West Femto Maximum Sensitivity Substrate (FEMTO; Thermo Fisher Scientific, MA, USA). The images were digitalized using a ChemiDoc MP system (Bio-Rad, Hercules, CA, USA). Protein content was expressed as the mean of the duplicates and was presented in arbitrary units related to human standards normalized to the average of all samples loaded on the same gel.

TABLE 1 | Overview of antibodies used for western blotting.

Primary antibody	Function	Supplier	Catalog No.	Dilution of primary antibody	Blocking agent	Secondary antibody	Dilution of secondary antibody	Detection agent
ERα	Receptor that binds oestrogenic compounds	Cell Sig.	8644	1:1,000	5% Milk, TBST	Goat Anti-Rabbit IgG	1:5,000	ECL
ERβ	Receptor that binds oestrogenic compounds	R&D Systems	MAP-7106	1:500	TBST	Goat Anti-Mouse IgG	1:5,000	ECL
ERRα	Estrogen-related receptor that can activate Estrogen Response Elements. Oestrogenic compounds can't be bound to ERRs	Abcam	76223	1:750	2% Milk, TBST	Goat Anti-Rabbit IgG	1:5,000	ECL
eNOS	Enzyme that is protective of the cardiovascular system, through the production of NO	Abcam	5589	1:10,00	5% Fish, TBST	Goat Anti-Rabbit IgG	1:5,000	FEMTO
PGI2S	Enzyme that produces prostacyclin, which is a vasodilatory compound and plays an important role in cardiovascular disease	Abcam	23668	1:300	3% BSA, TBST	Goat Anti-Rabbit IgG	1:5,000	ECL
SOD2	Transforms toxic superoxide, a product of the mitochondrial electron transport chain, to hydrogen peroxide	Merck Millipore	06-984	1:5,000	2% Milk, TBST	Goat Anti-Rabbit IgG	1:5,000	ECL
FLK1	Receptor with high affinity for VEGF, which mediates endothelial growth	Santa Cruz	393163	1:300	3% Fish, TBST	Goat Anti-Mouse IgG	1:5,000	ECL
CD31	Marker of endothelial cell differentiation	R&D Systems	AF806	1:250	2% Milk, TBST	Rabbit anti-sheep IgG	1:5,000	FEMTO

Figure 7-2: Western blotting antibodies.

ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; ERRα, estrogen related-receptor alpha; eNOS, endothelial nitric oxide synthase; PGI2S, prostacylin synthase; SOD2, superoxide dismutase; FLK1, vascular endothelial growth αβ α TM factor receptor 2; CD31, cluster of differentiation 31; BSA, bovine serum albumin; TBST, Tris-buffered saline tween; ECL, Luminata Forte; FEMTO, SuperSignal West Femto Maximum Sensitivity Substrate.

7.3.7 Statistical Analysis

The statistical analyses were performed using RStudio (R-Studio, Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria). Graphs were made in GraphPad Prism (GraphPad, Version 8.4.3, San Diego, CA, USA). An a priori power calculation was made for the primary outcome; a change in plasma VCAM-1 levels, based on previous data from our laboratory (Gliemann et al. 2013). For a power of 0.8 and an alpha value of 0.05 n = 9 subjects were estimated to be required. Paired t-tests were used to compare

the placebo and fermented red clover extract interventions. There was no observed order effect of the intervention (p = 0.994). Data are presented as mean ± SD. Individual values are shown in figures. Statistical significance was achieved if $p < 0.05$. Effect sizes (d) were calculated and reported for each parameter by dividing the mean of the differences by the standard deviation of the differences.

7.4 Results

7.4.1 Plasma Markers of Vascular Inflammation

Plasma levels of VCAM-1 were significantly reduced by ~5.4% following fermented red clover extract compared to placebo (503.2 ± 84.4 vs. 534.8 ± 96.8 ng mL^{-1}; p = 0.0321; *d* = 0.7; Figure 7-3A). However, there was no statistically significant effect of fermented red clover extract supplementation compared to placebo on ICAM-1 (628.2 ± 84.2 vs. 641.8 ± 93.2 ng mL^{-1}; p = 0.1310; *d* = 0.4; Figure 7-3B), SAA (3732.4 ± 1927.6 vs. 3894.2 ± 2021.3 ng mL^{-1}; p = 0.5877; *d* = -0.1; Figure 7-3C), or CRP (1656.4 ± 1354.4 vs. 1620.5 ± 1311.9 ng mL^{-1}; p = 0.2536; *d* = 0.2; Figure 7-3D).

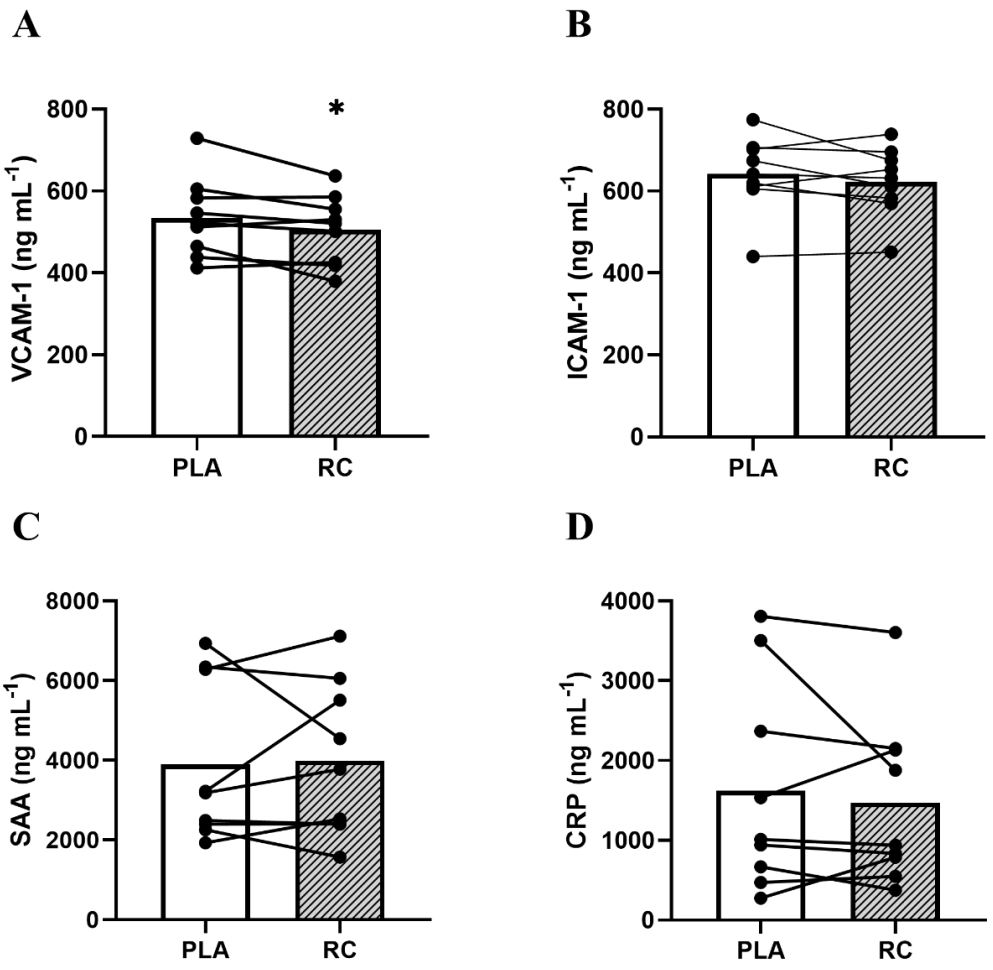

Figure 7-3: Markers of vascular inflammation.

Levels of inflammatory markers in plasma samples obtained from early post-menopausal women following two weeks of twice daily supplementation with placebo (PLA) or fermented red clover extract (RC) (n = 9). **(A)** Vascular cell adhesion molecule 1 (VCAM-1), **(B)** intracellular adhesion molecule 1 (ICAM-1), **(C)** serum amyloid A (SAA), and **(D)** C reactive protein (CRP). *, indicates $p < 0.05$.

7.4.2 Skeletal Muscle Oestrogen and Oestrogen Related-Receptor Content

The western blotting analysis showed no statistically significant effect of fermented red clover extract supplementation compared to placebo on ERα (0.89 ± 0.48 vs. 1.10 ± 0.76 AU; $p = 0.3170$; $d = 0.2$; Figure 7-4A), ERβ (0.99 ± 0.18 vs. 0.97 ± 0.30; $p = 0.5545$; $d = 0.0$; Figure 7-4B), and ERRα (0.95 ± 0.53 vs. 0.97 ± 0.51; $p = 0.7025$; $d = -0.2$; Figure 7-4C) protein content.

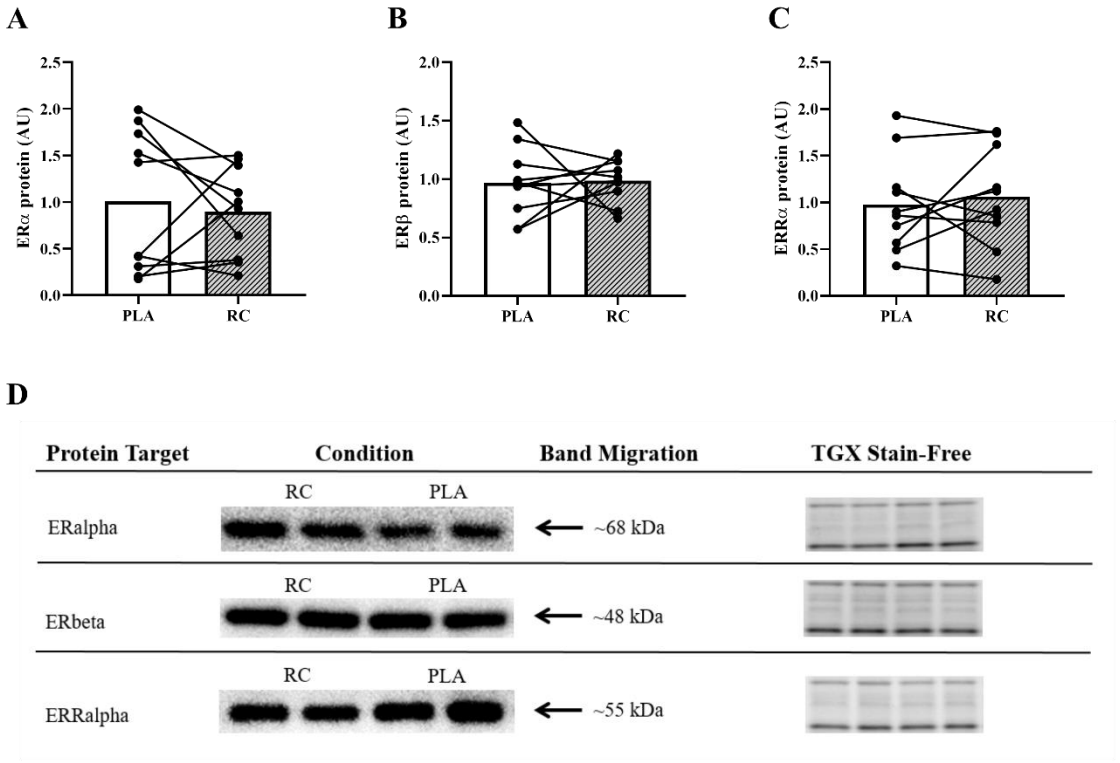

Figure 7-4: Protein expression of estrogen and estrogen-related receptor alpha.

Protein expression of oestrogen and oestrogen-related receptors in skeletal muscle from early post-menopausal women following two weeks of twice daily supplementation with placebo (PLA) or fermented red clover extract (RC) (n = 10). **(A)** Oestrogen receptor alpha (ERα), **(B)** Oestrogen receptor beta (ERβ), and **(C)** Oestrogen-related receptor alpha (ERRα). **(D)** Representative western blots and the corresponding TGX stain-free images.

7.4.3 Skeletal Muscle Protein Content of Enzymes

There was no statistically significant effect of fermented red clover extract supplementation compared to placebo on eNOS (1.05 ± 0.33 vs. 1.02 ± 0.42; p = 0.5837; *d* = -0.1; Figure 7-5A), PGI_2S (0.90 ± 0.50 vs. 1.08 ± 0.63; p = 0.2056; *d* = 0.3; Figure 7-5B), and SOD2 (0.99 ± 0.52 vs. 0.93± 0.51; p = 0.7060; *d* = -0.2; Figure 7-5C) protein content.

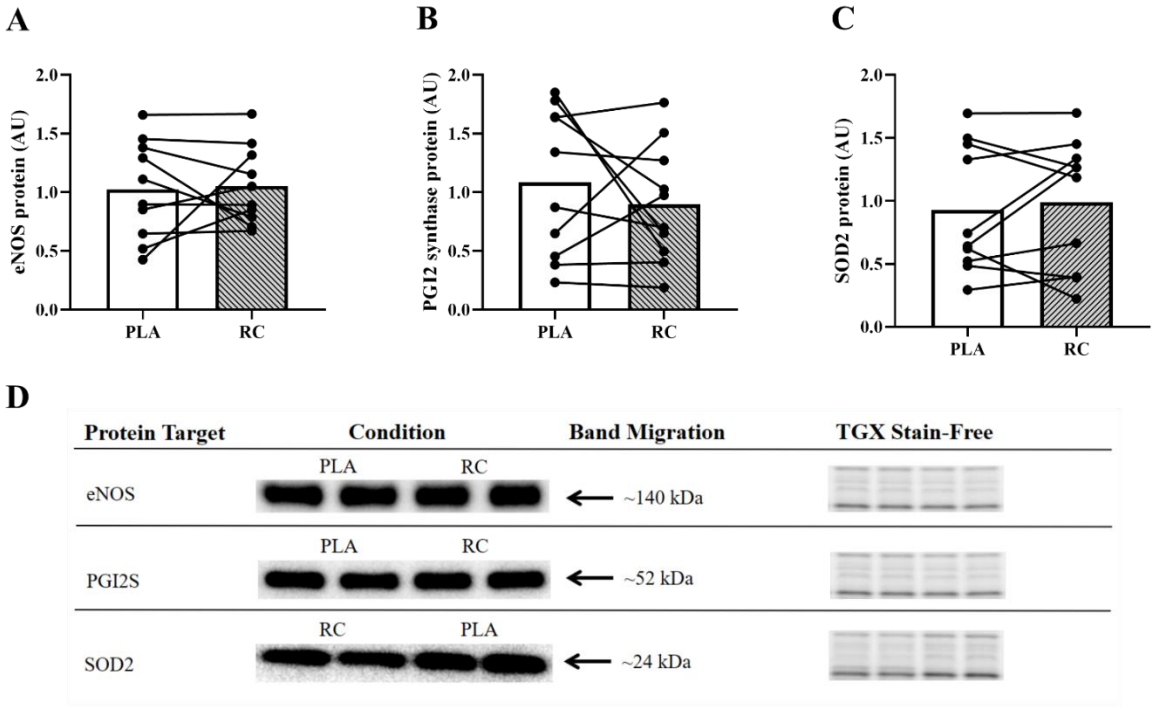

Figure 7-5:Protein expression related to vascular function.

Expression of proteins associated with vasodilatory capacity in skeletal muscle from early post-menopausal women following two weeks of twice daily supplementation with placebo (PLA) or fermented red clover extract (RC) (n = 10). **(A)** Endothelial nitric oxide synthase (eNOS), **(B)** prostacyclin synthase (PGI2S), and **(C)** superoxide dismutase 2 (SOD2). **(D)** Representative western blots and the corresponding TGX stain-free images.

7.4.4 Skeletal Muscle Content of Angiogenic Proteins

There was no statistically significant effect of fermented red clover extract supplementation compared to placebo on VEGFR2 (0.96 ± 0.22 vs. 1.06 ± 0.21; p = 0.1021; *d* = 0.4; Figure 7-6A) and CD31 (1.08 ± 0.61 vs. 1.07 ± 0.52; p = 0.5162; *d* = 0.0; Figure 7-6B) protein content.

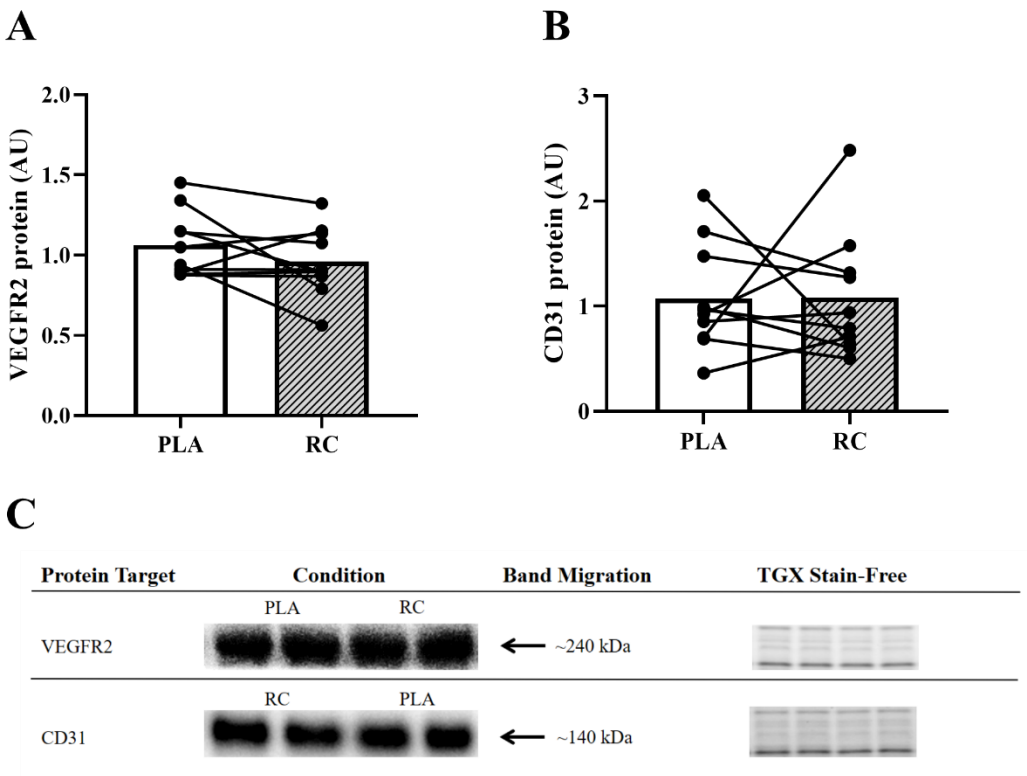

Figure 7-6: Protein expression related to angiogenesis.

Expression of proteins associated with angiogenic potential in skeletal muscle from early post-menopausal women following two weeks of twice daily supplementation with placebo (PLA) or fermented red clover extract (RC) (n = 10). **(A)** Vascular endothelial growth factor receptor 2 (VEGFR2) and **(B)** cluster of differentiation 31 (CD31). **(C)** Representative western blots and the corresponding TGX stain-free images.

7.5 Discussion

The main objective of this study was to determine whether two weeks of twice daily supplementation with fermented red clover extract could improve markers of vascular health in the plasma and skeletal muscle of early post-menopausal women. The primary finding was a reduction in circulating VCAM-1 levels following two weeks of fermented red clover extract supplementation compared to placebo. Fermented red clover extract supplementation did not lead to significant alterations in any of the other plasma markers

of vascular inflammation or skeletal muscle protein content of oestrogen and oestrogen-related receptors as well as enzymes related to vascular function and angiogenesis.

7.5.1 Plasma Markers of Vascular Inflammation

Vascular inflammation is characterized by the activation of cell adhesion molecules, which contributes to the development of atherosclerosis (Szmitko et al., 2003) and is inversely correlated with oestrogen status (Caulin-Glaser et al., 1996; Colacurci et al., 2005; Abu-Taha et al., 2009). In this study, we measured plasma levels of VCAM-1, ICAM-1, SAA, and CRP as key markers of vascular inflammation and cell adhesion in response to short-term fermented red clover extract supplementation in early post-menopausal women. Corroborating our hypothesis, we found a statistically significant reduction (~5.4%), with a moderate-to-large effect size (d = 0.7), in the VCAM-1 level following short-term fermented red clover extract supplementation (Figure 7-3A). However, there were no statistically significant changes in CRP, SAA, or ICAM-1 levels (Figure 7-3B-D). Although, ICAM-1 had a small-to-moderate effect size (d = 0.4) and CRP had a small effect size (d = 0.2), suggesting possible reductions in these inflammatory markers following fermented red clover extract supplementation compared to placebo. A greater depth of research is required to validate these potential effects. In response to elevated circulating cytokines, VCAM-1 plays a crucial role in mediating immune cell adhesion to the vascular endothelium (Szmitko et al., 2003). Importantly, oestrogen and phytoestrogens can elicit anti-inflammatory effects, thereby reducing vascular inflammation (Colacurci et al., 2005; Hall et al., 2005; Lebon et al., 2014). We found that VCAM-1 was the only inflammatory marker that was significantly improved following fermented red clover extract

supplementation. Interestingly, previous cell culture work has shown that oestrogen elicits more robust beneficial effects on cytokine-stimulated VCAM-1 expression compared to other markers of vascular inflammation, such as ICAM-1 (Hou and Pei, 2015), which may explain the findings of the current study. In line with our findings, a previous investigation by (Teede et al., 2003) found a decline in plasma VCAM-1 in parallel with a reduction in arterial stiffness after 6 weeks of supplementation with isolated formononetin-rich isoflavones in healthy men and post-menopausal women. The lowering of VCAM-1 levels may indicate reduced atherosclerosis, as the level of circulating cell adhesion molecules is positively correlated with the degree of atherosclerosis in humans (Rohde et al., 1998). The mechanisms underpinning the statistically significant reduction in VCAM-1 levels remains to be elucidated. Specifically, it is unclear whether isoflavones elicit a direct anti-inflammatory effect or if they facilitate anti-inflammatory signalling cascades, such as the promotion of NO or PGI_2 release from the vascular endothelium.

7.5.2 Regulation of Skeletal Muscle Oestrogen Receptors and Microvascular Proteins

A depth of research has emerged demonstrating the importance of the oestrogen receptor-mediated pathways for cardiovascular and metabolic health (Tiidus et al., 2013; Ikeda et al., 2019). Notably, in post-menopausal women, the impaired interaction of oestrogen with its receptors in the skeletal muscle microvasculature may play a vital role in the development of cardiovascular and metabolic diseases. In this study, we sought to determine whether short-term fermented red clover extract supplementation could mitigate some of these impairments.

7.5.2.1 Oestrogen Receptor Content

A large proportion of the beneficial effects of oestrogen are mediated by the activation of signalling cascades when oestrogen binds to its steroid receptors (Kim et al., 2008). Recent research has shown that menopause is associated with a decline in skeletal muscle oestrogen receptor content (Park et al., 2017b) and the tendency towards a higher ratio of ERβ to ERα (Park et al., 2017a) Together, these factors have been linked to adverse cardiovascular outcomes (Park et al., 2017b). Importantly, in rodent models of menopause, oestrogen receptor content can be increased in response to recurring stimuli that utilize these pathways, such as hormone replacement therapy (Baltgalvis et al., 2010) and endurance exercise training (Lemoine et al., 2002). Moreover, the ratio of ERβ to ERα can be restored to pre-menopausal levels following treatment with oestrogen (Sakaguchi et al., 2003). However, in the current study, we did not observe a change in the expression of oestrogen receptor content following fermented red clover extract supplementation as assessed by ERα and ERβ (Figure 7-4A-B). Oxfeldt et al. (2020) also found no statistically significant change in ERβ protein content in skeletal muscle following fermented red clover extract supplementation compared to placebo. Importantly, ERβ protein content was determined using two different antibodies (Figure 7-2), highlighting the reproducibility of our findings.

7.5.2.2 Protein Content of Enzymes, Angiogenic Markers, and ERRα

Previous studies have shown that within the first few years after menopause women have reduced vascular function (Nyberg et al. 2017) and angiogenic potential (Olsen et al. 2020). Although exercise training interventions have been shown to enhance markers of

vascular function (Seals et al., 2019) and angiogenesis (Izzicupo et al., 2017) in post-menopausal women, no study to date has explored the effects of phytoestrogen supplementation on such markers. To investigate the effects of fermented red clover extract supplementation on skeletal muscle enzymes, we measured eNOS and PGI_2S, which are responsible for producing the vasodilators NO and PGI_2, respectively (Mitchell et al., 2008). Additionally, we measured SOD2 as it is an antioxidant enzyme that can facilitate NO bioavailability (Fukai and Ushio-Fukai, 2011). Contrary to our initial hypothesis, we did not find any statistically significant changes in the protein content of these skeletal muscle enzymes following fermented red clover extract supplementation compared to placebo (Figure 7-5A-C). However, following fermented red clover extract supplementation compared to placebo, we observed small effect sizes for PGI_2S (d = 0.3) and SOD2 (d = -0.2), suggesting a potential decrease and increase in protein content, respectively. More research is required to validate and draw conclusions regarding these potential effects. Our results are in contrast with recent findings in human endothelial cells, which demonstrated that red clover elicits increased eNOS expression, enhanced NO production, and lowered reactive oxygen species production (Jeon et al., 2020). Although we did not observe a statistically significant increase in eNOS or PGI_2S protein expression, it is possible that fermented red clover extract supplementation improved the activity of these enzymes and thereby, contributed to the observed reduction in vascular inflammation in this study (Best et al., 1998; Colacurci et al., 2005). Evidently, future work is required to tease apart these mechanisms. Moreover, VEGFR2 and CD31 were measured to assess the effects of fermented red clover extract supplementation on

angiogenic markers in skeletal muscle. We did not observe any statistically significant changes in angiogenic proteins following fermented red clover extract supplementation compared to placebo (Figure 7-6A-B). However, we did observe a small-to-moderate effect on VEGFR2 protein content (*d* = 0.4), suggesting a possible decrease following fermented red clover extract supplementation compared to placebo. A greater body of evidence is required to support or refute this potential effect. Lastly, we measured ERRα protein content, as it is a non-steroid receptor that is abundant in skeletal muscle and exclusively expressed in endothelial cells (Likhite et al., 2019). Importantly, the isoflavones in fermented red clover extract have been shown to behave as agonists that promote ERRα activity (Suetsugi et al., 2003), which could potentially increase eNOS expression (Sumi and Ignarro, 2003) as well as angiogenesis (Likhite et al., 2019). However, we did not see any change in ERRα protein content following fermented red clover extract supplementation (Figure 7-4C).

7.5.3 Study Limitations

Ten subjects were included in the cross over design and it cannot be excluded that the power was too low for some of the variables measured. In addition, the two-week fermented red clover extract intervention may have been too short to elicit an optimal effect on the plasma inflammatory profile and skeletal muscle proteins. However, our intervention period was selected based on the premise that as little as one week of oestrogen therapy has been shown to induce significant changes in skeletal muscle protein content (Park et al., 2019). To limit the invasive procedures for the participants, plasma and skeletal muscle samples were only obtained at the end of the 14-day

interventions and, therefore, we do not have baseline measurements. However, the study was of a randomized, crossover design and we did not observe an order effect, which minimizes the likelihood of this limitation. Moreover, we did not determine formononetin and biochanin A levels via high performance liquid chromatography (Collison, 2008). Therefore, it is unclear how much of the fermented red clover extract was successfully delivered to the skeletal muscle. Lastly, the study participants, though sedentary, were healthy and since this study did not include a functional vascular measure, it is possible that the cardiovascular health of the participants may have been too high to see beneficial effects with fermented red clover extract supplementation.

7.5.4 Future Directions

The findings from this study have unearthed novel evidence regarding the cardiovascular benefits of fermented red clover extract, which support the known therapeutic potential of fermented red clover extract in relieving menopausal symptoms (Lambert et al., 2017a), promoting bone health (Thorup et al., 2015), and minimizing skeletal muscle breakdown (Oxfeldt et al., 2020) in post-menopausal women. Future investigations are required to elucidate the optimal dosing regime and mechanisms of action, together with reporting of potential side effects. Importantly, future investigations should consider increasing the duration of supplementation to optimize the therapeutic effects on cardiovascular and potentially metabolic health. Moreover, future investigations should consider combining fermented red clover extract supplementation with exercise training to determine whether this strategy can be used to maximize cardiovascular and metabolic benefits in post-menopausal women. Lastly, the optimal timing after menopause for

initiation of fermented red clover extract supplementation should be evaluated, as a growing body of evidence suggests therapeutic interventions (e.g. HRT or aerobic exercise training) are more successful in early post-menopausal women compared to late post-menopausal women (Gliemann and Hellsten, 2019).

7.5.5 Conclusions

This study provides the first evidence that as little as two weeks of twice-daily supplementation with fermented red clover extract reduces VCAM-1 expression and significantly improves vascular inflammation in early post-menopausal women.

7.6 References

Abu-Taha, M., Rius, C., Hermenegildo, C., Noguera, I., Cerda-Nicolas, J.M., Issekutz, A.C., et al. (2009). Menopause and ovariectomy cause a low grade of systemic inflammation that may be prevented by chronic treatment with low doses of estrogen or losartan. J Immunol 183(2), 1393-1402. doi: 10.4049/jimmunol.0803157.

Baltgalvis, K.A., Greising, S.M., Warren, G.L., and Lowe, D.A. (2010). Estrogen regulates estrogen receptors and antioxidant gene expression in mouse skeletal muscle. PLoS One 5(4), e10164. doi: 10.1371/journal.pone.0010164.

Barrett-Connor, E. (1997). Sex differences in coronary heart disease. Why are women so superior? The 1995 Ancel Keys Lecture. Circulation 95(1), 252-264. doi: 10.1161/01.cir.95.1.252.

Best, P.J., Berger, P.B., Miller, V.M., and Lerman, A. (1998). The effect of estrogen replacement therapy on plasma nitric oxide and endothelin-1 levels in postmenopausal women. Ann Intern Med 128(4), 285-288. doi: 10.7326/0003-4819-128-4-199802150-00006.

Caulin-Glaser, T., Watson, C.A., Pardi, R., and Bender, J.R. (1996). Effects of 17beta-estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Invest 98(1), 36-42. doi: 10.1172/JCI118774.

Colacurci, N., Chiantera, A., Fornaro, F., de Novellis, V., Manzella, D., Arciello, A., et al. (2005). Effects of soy isoflavones on endothelial function in healthy postmenopausal women. Menopause 12(3), 299-307. doi: 10.1097/01.gme.0000147017.23173.5b.

Collison, M.W. (2008). Determination of total soy isoflavones in dietary supplements, supplement ingredients, and soy foods by high-performance liquid chromatography with ultraviolet detection: collaborative study. J AOAC Int 91(3), 489-500.

Couse, J.F., Lindzey, J., Grandien, K., Gustafsson, J.A., and Korach, K.S. (1997). Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 138(11), 4613-4621. doi: 10.1210/endo.138.11.5496.

Dubey, R.K., and Jackson, E.K. (2001). Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. Am J Physiol Renal Physiol 280(3), F365-388. doi: 10.1152/ajprenal.2001.280.3.F365.

Fukai, T., and Ushio-Fukai, M. (2011). Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal 15(6), 1583-1606. doi: 10.1089/ars.2011.3999.

Garcia-Martinez, M.C., Hermenegildo, C., Tarin, J.J., and Cano, A. (2003). Phytoestrogens increase the capacity of serum to stimulate prostacyclin release in human endothelial cells. Acta Obstet Gynecol Scand 82(8), 705-710. doi: 10.1080/j.1600-0412.2003.00178.x.

Gliemann, L., and Hellsten, Y. (2019). The exercise timing hypothesis: can exercise training compensate for the reduction in blood vessel function after menopause if timed right? J Physiol 597(19), 4915-4925. doi: 10.1113/JP277056.

Gliemann, L., Schmidt, J.F., Olesen, J., Bienso, R.S., Peronard, S.L., Grandjean, S.U., et al. (2013). Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. J Physiol 591(20), 5047-5059. doi: 10.1113/jphysiol.2013.258061.

Grady, D., Rubin, S.M., Petitti, D.B., Fox, C.S., Black, D., Ettinger, B., et al. (1992). Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 117(12), 1016-1037. doi: 10.7326/0003-4819-117-12-1016.

Hall, W.L., Vafeiadou, K., Hallund, J., Bugel, S., Koebnick, C., Reimann, M., et al. (2005). Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. Am J Clin Nutr 82(6), 1260-1268; quiz 1365-1266. doi: 10.1093/ajcn/82.6.1260.

Hodges, N.A., Suarez-Martinez, A.D., and Murfee, W.L. (2018). Understanding angiogenesis during aging: opportunities for discoveries and new models. J Appl Physiol (1985) 125(6), 1843-1850. doi: 10.1152/japplphysiol.00112.2018.

Hodis, H.N., Mack, W.J., Henderson, V.W., Shoupe, D., Budoff, M.J., Hwang-Levine, J., et al. (2016). Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol. N Engl J Med 374(13), 1221-1231. doi: 10.1056/NEJMoa1505241.

Hoier, B., Nordsborg, N., Andersen, S., Jensen, L., Nybo, L., Bangsbo, J., et al. (2012). Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. J Physiol 590(3), 595-606. doi: 10.1113/jphysiol.2011.216135.

Horn-Ross, P.L., John, E.M., Canchola, A.J., Stewart, S.L., and Lee, M.M. (2003). Phytoestrogen intake and endometrial cancer risk. J Natl Cancer Inst 95(15), 1158-1164. doi: 10.1093/jnci/djg015.

Hou, X., and Pei, F. (2015). Estradiol Inhibits Cytokine-Induced Expression of VCAM-1 and ICAM-1 in Cultured Human Endothelial Cells Via AMPK/PPARalpha Activation. Cell Biochem Biophys 72(3), 709-717. doi: 10.1007/s12013-015-0522-y.

Howes, J.B., Tran, D., Brillante, D., and Howes, L.G. (2003). Effects of dietary supplementation with isoflavones from red clover on ambulatory blood pressure and endothelial function in postmenopausal type 2 diabetes. Diabetes Obes Metab 5(5), 325-332. doi: 10.1046/j.1463-1326.2003.00282.x.

Ikeda, K., Horie-Inoue, K., and Inoue, S. (2019). Functions of estrogen and estrogen receptor signaling on skeletal muscle. J Steroid Biochem Mol Biol 191, 105375. doi: 10.1016/j.jsbmb.2019.105375.

Iorga, A., Cunningham, C.M., Moazeni, S., Ruffenach, G., Umar, S., and Eghbali, M. (2017). The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. Biol Sex Differ 8(1), 33. doi: 10.1186/s13293-017-0152-8.

Izzicupo, P., D'Amico, M.A., Di Blasio, A., Napolitano, G., Nakamura, F.Y., Di Baldassarre, A., et al. (2017). Aerobic Training Improves Angiogenic Potential Independently of Vascular Endothelial Growth Factor Modifications in Postmenopausal Women. Front Endocrinol (Lausanne) 8, 363. doi: 10.3389/fendo.2017.00363.

Jeon, S.Y., Kim, M.R., Lee, E.O., Jeon, B.H., Lee, J.J., and Lee, Y.C. (2020). Effect of a new herbal composition comprised of red clover and hop extract on human endothelial cell damage and vasorelaxant activity. J Food Biochem 44(8), e13314. doi: 10.1111/jfbc.13314.

Kim, K.H., Moriarty, K., and Bender, J.R. (2008). Vascular cell signaling by membrane estrogen receptors. Steroids 73(9-10), 864-869. doi: 10.1016/j.steroids.2008.01.008.

Kim, M.R., Kim, H.J., Yu, S.H., Lee, B.S., Jeon, S.Y., Lee, J.J., et al. (2020). Combination of Red Clover and Hops Extract Improved Menopause Symptoms in an Ovariectomized Rat Model. Evid Based Complement Alternat Med 2020, 7941391. doi: 10.1155/2020/7941391.

Lambert, M.N.T., Thorup, A.C., Hansen, E.S.S., and Jeppesen, P.B. (2017a). Combined Red Clover isoflavones and probiotics potently reduce menopausal vasomotor symptoms. PLoS One 12(6), e0176590. doi: 10.1371/journal.pone.0176590.

Lambert, M.N.T., Thybo, C.B., Lykkeboe, S., Rasmussen, L.M., Frette, X., Christensen, L.P., et al. (2017b). Combined bioavailable isoflavones and probiotics improve bone status and estrogen metabolism in postmenopausal osteopenic women: a

randomized controlled trial. Am J Clin Nutr 106(3), 909-920. doi: 10.3945/ajcn.117.153353.

Lebon, J., Riesco, E., Tessier, D., and Dionne, I.J. (2014). Additive effects of isoflavones and exercise training on inflammatory cytokines and body composition in overweight and obese postmenopausal women: a randomized controlled trial. Menopause 21(8), 869-875. doi: 10.1097/GME.0000000000000177.

Lemeziene, N., Padarauskas, A., Butkute, B., Ceseviciene, J., Taujenis, L., Norkeviciene, E., et al. (2015). The concentration of isoflavones in red clover (Trifolium pratense L.) at flowering stage. Zemdirbyste-Agriculture 102(4), 443-448. doi: DOI 10.13080/z-a.2015.102.057.

Lemoine, S., Granier, P., Tiffoche, C., Berthon, P.M., Thieulant, M.L., Carre, F., et al. (2002). Effect of endurance training on oestrogen receptor alpha expression in different rat skeletal muscle type. Acta Physiol Scand 175(3), 211-217. doi: 10.1046/j.1365-201X.2002.00992.x.

Lerner, D.J., and Kannel, W.B. (1986). Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am Heart J 111(2), 383-390.

Li, S.H., Liu, X.X., Bai, Y.Y., Wang, X.J., Sun, K., Chen, J.Z., et al. (2010). Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. Am J Clin Nutr 91(2), 480-486. doi: 10.3945/ajcn.2009.28203.

Likhite, N., Yadav, V., Milliman, E.J., Sopariwala, D.H., Lorca, S., Narayana, N.P., et al. (2019). Loss of Estrogen-Related Receptor Alpha Facilitates Angiogenesis in Endothelial Cells. Mol Cell Biol 39(5). doi: 10.1128/MCB.00411-18.

Mainini, G., Torella, M., Di Donna, M.C., Esposito, E., Ercolano, S., Correa, R., et al. (2013). Nonhormonal management of postmenopausal women: effects of a red clover based isoflavones supplementation on climacteric syndrome and cardiovascular risk serum profile. Clin Exp Obstet Gynecol 40(3), 337-341.

Mendelsohn, M.E., and Karas, R.H. (1999). The protective effects of estrogen on the cardiovascular system. N Engl J Med 340(23), 1801-1811. doi: 10.1056/NEJM199906103402306.

Messerli, F.H., Garavaglia, G.E., Schmieder, R.E., Sundgaard-Riise, K., Nunez, B.D., and Amodeo, C. (1987). Disparate cardiovascular findings in men and women with essential hypertension. Ann Intern Med 107(2), 158-161.

Mikkola, T.S., Gissler, M., Merikukka, M., Tuomikoski, P., and Ylikorkala, O. (2013). Sex differences in age-related cardiovascular mortality. PLoS One 8(5), e63347. doi: 10.1371/journal.pone.0063347.

Miller, V.M., Black, D.M., Brinton, E.A., Budoff, M.J., Cedars, M.I., Hodis, H.N., et al. (2009). Using basic science to design a clinical trial: baseline characteristics of women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). J Cardiovasc Transl Res 2(3), 228-239. doi: 10.1007/s12265-009-9104-y.

Mitchell, J.A., Ali, F., Bailey, L., Moreno, L., and Harrington, L.S. (2008). Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. Exp Physiol 93(1), 141-147. doi: 10.1113/expphysiol.2007.038588.

Maas, A.H., and Franke, H.R. (2009). Women's health in menopause with a focus on hypertension. Neth Heart J 17(2), 68-72. doi: 10.1007/BF03086220.

Naftolin, F., Taylor, H.S., Karas, R., Brinton, E., Newman, I., Clarkson, T.B., et al. (2004). The Women's Health Initiative could not have detected cardioprotective effects of starting hormone therapy during the menopausal transition. Fertil Steril 81(6), 1498-1501. doi: 10.1016/j.fertnstert.2004.02.095.

Nestel, P.J., Pomeroy, S., Kay, S., Komesaroff, P., Behrsing, J., Cameron, J.D., et al. (1999). Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. J Clin Endocrinol Metab 84(3), 895-898. doi: 10.1210/jcem.84.3.5561.

Novella, S., Dantas, A.P., Segarra, G., Medina, P., and Hermenegildo, C. (2012). Vascular Aging in Women: is Estrogen the Fountain of Youth? Front Physiol 3, 165. doi: 10.3389/fphys.2012.00165.

Olsen, L.N., Hoier, B., Hansen, C.V., Leinum, M., Carter, H.H., Jorgensen, T.S., et al. (2020). Angiogenic potential is reduced in skeletal muscle of aged women. J Physiol 598(22), 5149-5164. doi: 10.1113/JP280189.

Oxfeldt, M., Dalgaard, L.B., Risikesan, J., Johansen, F.T., and Hansen, M. (2020). Influence of Fermented Red Clover Extract on Skeletal Muscle in Early Postmenopausal Women: A Double-Blinded Cross-Over Study. Nutrients 12(11). doi: 10.3390/nu12113587.

Park, Y.M., Erickson, C., Bessesen, D., Van Pelt, R.E., and Cox-York, K. (2017a). Age- and menopause-related differences in subcutaneous adipose tissue estrogen receptor mRNA expression. Steroids 121, 17-21. doi: 10.1016/j.steroids.2017.03.001.

Park, Y.M., Keller, A.C., Runchey, S.S., Miller, B.F., Kohrt, W.M., Van Pelt, R.E., et al. (2019). Acute estradiol treatment reduces skeletal muscle protein breakdown markers in

early- but not late-postmenopausal women. Steroids 146, 43-49. doi: 10.1016/j.steroids.2019.03.008.

Park, Y.M., Pereira, R.I., Erickson, C.B., Swibas, T.A., Kang, C., and Van Pelt, R.E. (2017b). Time since menopause and skeletal muscle estrogen receptors, PGC-1alpha, and AMPK. Menopause 24(7), 815-823. doi: 10.1097/GME.0000000000000829.

Pfitscher, A., Reiter, E., and Jungbauer, A. (2008). Receptor binding and transactivation activities of red clover isoflavones and their metabolites. J Steroid Biochem Mol Biol 112(1-3), 87-94. doi: 10.1016/j.jsbmb.2008.08.007.

Pilsakova, L., Riecansky, I., and Jagla, F. (2010). The physiological actions of isoflavone phytoestrogens. Physiol Res 59(5), 651-664. doi: 10.33549/physiolres.931902.

Rohde, L.E., Lee, R.T., Rivero, J., Jamacochian, M., Arroyo, L.H., Briggs, W., et al. (1998). Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. Arterioscler Thromb Vasc Biol 18(11), 1765-1770. doi: 10.1161/01.atv.18.11.1765.

Sakaguchi, H., Fujimoto, J., Aoki, I., and Tamaya, T. (2003). Expression of estrogen receptor alpha and beta in myometrium of premenopausal and postmenopausal women. Steroids 68(1), 11-19. doi: 10.1016/s0039-128x(02)00111-3.

Sathyapalan, T., Aye, M., Rigby, A.S., Thatcher, N.J., Dargham, S.R., Kilpatrick, E.S., et al. (2018). Soy isoflavones improve cardiovascular disease risk markers in women during the early menopause. Nutr Metab Cardiovasc Dis 28(7), 691-697. doi: 10.1016/j.numecd.2018.03.007.

Seals, D.R., Nagy, E.E., and Moreau, K.L. (2019). Aerobic exercise training and vascular function with ageing in healthy men and women. J Physiol 597(19), 4901-4914. doi: 10.1113/JP277764.

Simoncini, T., Garibaldi, S., Fu, X.D., Pisaneschi, S., Begliuomini, S., Baldacci, C., et al. (2008). Effects of phytoestrogens derived from red clover on atherogenic adhesion molecules in human endothelial cells. Menopause 15(3), 542-550. doi: 10.1097/gme.0b013e318156f9d6.

Squadrito, F., Altavilla, D., Crisafulli, A., Saitta, A., Cucinotta, D., Morabito, N., et al. (2003). Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. Am J Med 114(6), 470-476. doi: 10.1016/s0002-9343(03)00059-7.

Suetsugi, M., Su, L., Karlsberg, K., Yuan, Y.C., and Chen, S. (2003). Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors. Mol Cancer Res 1(13), 981-991.

Sumi, D., and Ignarro, L.J. (2003). Estrogen-related receptor alpha 1 up-regulates endothelial nitric oxide synthase expression. Proc Natl Acad Sci U S A 100(24), 14451-14456. doi: 10.1073/pnas.2235590100.

Szmitko, P.E., Wang, C.H., Weisel, R.D., de Almeida, J.R., Anderson, T.J., and Verma, S. (2003). New markers of inflammation and endothelial cell activation: Part I. Circulation 108(16), 1917-1923. doi: 10.1161/01.CIR.0000089190.95415.9F.

Taddei, S., Virdis, A., Ghiadoni, L., Mattei, P., Sudano, I., Bernini, G., et al. (1996). Menopause is associated with endothelial dysfunction in women. Hypertension 28(4), 576-582.

Teede, H.J., McGrath, B.P., DeSilva, L., Cehun, M., Fassoulakis, A., and Nestel, P.J. (2003). Isoflavones reduce arterial stiffness: a placebo-controlled study in men and postmenopausal women. Arterioscler Thromb Vasc Biol 23(6), 1066-1071. doi: 10.1161/01.ATV.0000072967.97296.4A.

Thorup, A.C., Lambert, M.N., Kahr, H.S., Bjerre, M., and Jeppesen, P.B. (2015). Intake of Novel Red Clover Supplementation for 12 Weeks Improves Bone Status in Healthy Menopausal Women. Evid Based Complement Alternat Med 2015, 689138. doi: 10.1155/2015/689138.

Tiidus, P.M., Lowe, D.A., and Brown, M. (2013). Estrogen replacement and skeletal muscle: mechanisms and population health. J Appl Physiol (1985) 115(5), 569-578. doi: 10.1152/japplphysiol.00629.2013.

Ting, Y., Jiang, Y., Ho, C.-T., and Huang, Q. (2014). Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals. Journal of Functional Foods 7, 112-128. doi: https://doi.org/10.1016/j.jff.2013.12.010.

CHAPTER 8 GENERAL DISCUSSION

This dissertation expanded the inclusion of older women in cardiovascular, exercise and nutraceutical research by evaluating the efficacy of alternatives to pharmaceutical therapies, such as exercise training and supplementation with fermented red clover extract, for promoting vascular health in postmenopausal women. This novel research program was the first to: **(1)** explore the effects of exercise training on the efficacy of *in-vitro* dual anti-platelet therapy (DAPT) in postmenopausal women (*Chapter 4*), **(2)** experimentally evaluate the *exercise timing hypothesis* with respect to vascular adaptations (*Chapter 6*), and **(3)** evaluate the efficacy of fermented red clover extract for promoting vascular health in postmenopausal women (*Chapter 7*).

8.1 Summary of Findings

This research program produced several findings that demonstrate the efficacy of short-term exercise training and fermented red clover interventions for improving vascular health in postmenopausal women, although these adaptations may be best achieved in the first few years after menopause. *Chapter 4* demonstrated that as little as 8 weeks of high-intensity exercise training can improve platelet prostacyclin sensitivity and potentiate the *in-vitro* DAPT response, suggesting it may be important to consider training status when prescribing DAPT to postmenopausal women. *Chapter 5* summarized the existing literature regarding exercise training and vascular health in postmenopausal women. The conclusions from this review provided compelling evidence in support of the *exercise timing hypothesis*, where cardiovascular adaptations to exercise training appear

to be more robust in women who are recently postmenopausal. Accordingly, in *Chapter 6*, we validated the *exercise timing hypothesis* for the first time in an experimental model. We showed that 8 weeks of exercise training significantly reduced thrombotic risk in recently postmenopausal women but not in late postmenopausal women. Based on the findings from *Chapters 5 and 6*, we chose to pursue a phytoestrogen supplementation study in recently postmenopausal women only. The findings from *Chapter 7* highlighted that as little as 2 weeks of supplementation with fermented red clover extract can improve markers of vascular inflammation in recently postmenopausal women. Taken together, the time for previously sedentary women to start taking control of their vascular health is now. In women around the menopausal transition, this can be achieved via regular physical activity or supplementation with fermented red clover extract. Importantly, if a woman has a sedentary life until the menopausal transition, it is not too late to start implementing alternative strategies, such as regular physical activity or supplementation with fermented red clover extract, to promote vascular health. Although, it is important to consider that it may be easier to achieve positive effects of these interventions if they are started closer to the menopausal transition, as opposed to several years after menopause.

8.2 Perspectives

8.2.1 Global Reproducibility

It is interesting to bring a geographical and cultural perspective into the interpretation of these results, where these studies were conducted in healthy postmenopausal women from Copenhagen, Denmark. Undoubtedly, around the globe there are extremely diverse

habits for participation in and adherence to regular physical activity, which is at least partially driven by lifestyle, values, and accessibility. For example, Denmark reports one of the highest physical activity and fitness levels in adults worldwide (Nauman et al., 2017). Conversely, only 50% of Canadian (Government of Canada, 2021) and 25% of American (Hyde et al., 2021) adults meet the physical activity recommendations. Interestingly, the prevalence of death from cardiovascular disease is inversely related to physical activity levels, whereby Denmark reports the lowest values (36.00 deaths per 100,000 people) followed by Canada (45.15 deaths per 100,000 people), and USA reports the highest (73.49 deaths per 100,000 people) (World Health Organization, 2022). Therefore, due to differences in modifiable lifestyle factors between countries, it is likely that the prevalence and severity of cardiovascular disease differs before and after the menopausal transition, which could produce divergent results in the studies included in this dissertation if they were replicated in other countries. This is considered in the future directions section.

8.2.2 Consideration of Responders versus Non-Responders

It is well-established that there is significant interindividual variability in human physiology (Chrzanowski-Smith et al., 2020). This may be attributed to genetic (Bouchard et al., 1999) as well as lifestyle factors, such as sleep (Samuels, 2009) and nutrition (Hawley et al., 2011). Importantly, these interindividual differences translate to the adaptive responses to exercise and nutritional interventions (Mann et al., 2014). For example, even the best controlled exercise training interventions will produce variable adaptations, since typical methodologies to prescribe exercise intensity (e.g., percentage

of HR_{max} or $\dot{V}O_{2max}$) inherently elicit different physiological stimuli between individuals (Mann et al., 2014; Scharhag-Rosenberger et al., 2010). A previous report showed that up to 20% of healthy individuals do not gain beneficial cardiovascular adaptations from a period of exercise training, termed “non-responders” (Timmons et al., 2010). However, Montero and Lundby (2017) demonstrated that “non-responders” to exercise training interventions do not exist in healthy cohorts. Instead, they propose that there is interindividual variability in the appropriate exercise training stimulus and/or duration of training required to elicit beneficial adaptations (Montero and Lundby, 2017). Therefore, it is possible that a longer high-intensity training intervention (i.e., >8 weeks) may have been necessary to promote beneficial adaptations in the postmenopausal women who were “non-responders” in Studies I and II. This is considered in the limitations and future directions sections. Though speculative, the explanation for observing “non-responders” after 8 weeks of high-intensity training may depend on factors such as circulating estrogen, contraction-mediated activation of ERRα, or inflammatory responses and recovery after training.

Similarly, in nutraceutical interventions, there is considerable interindividual variability in the bioavailability of the supplement, where factors like digestion and absorption of orally ingested treatments as well as accumulation in the circulation and interactions with the target tissue can impact the efficacy of the nutraceutical intervention (Wickham and Spriet, 2023). The development of pharmacokinetic profiles can help determine the optimal dosing strategy for nutraceutical interventions in the general population, including dosing and frequency of supplementation (Wickham and Spriet, 2023). Due to

the novelty of supplementation with fermented red clover extract, this valuable information is currently lacking. Therefore, in Study III, a greater depth of research is required to determine whether a longer supplementation protocol or different dosing regime is required to maximize the beneficial effects on vascular health. Moreover, it is interesting to consider whether individuals with higher ERα content have a greater capacity for beneficial adaptations to short-term supplementation with fermented red clover extract.

8.2.3 The Role of Other Sex Hormones

Finally, although the deleterious physiological effects of menopause are generally credited to the loss of endogenous estrogen, it is important to consider that endogenous progestogens also diminishes with menopause (Santoro and Randolph, 2011). Intriguingly, progesterone has been shown to lower blood pressure, inhibit coronary hyperreactivity, and act as a potent vasodilator (Thomas and Pang, 2013). Therefore, it is perhaps an oversimplification to discuss the vascular consequences of menopause with a sole focus on estrogen, and a greater depth of research is required to elucidate the physiological roles of progestogens as they relate to menopause.

8.3 Limitations

Although the limitations central to each study are discussed in the individual manuscripts, there are some overarching limitations to the studies included in the dissertation. First, a main limitation is that the intervention periods were short in duration (i.e., 8 weeks of exercise training and 2 weeks of supplementation with fermented red clover extract).

Although these interventions were long enough to facilitate positive changes to vascular health, it is likely that more robust changes would have been observed with a longer intervention (e.g., 12 weeks of exercise training and supplementation with fermented red clover extract). Another limitation of the studies included in this dissertation is the lack of activity and diet tracking throughout the interventions. Therefore, it is impossible to ascribe the physiological benefits solely to the exercise or fermented red clover extract interventions, as it is possible that some of the observed outcomes are due to additional changes in diet and exercise habits. The Hawthorne Effect is a known psychological phenomenon where individuals change their behaviours (e.g., dietary or exercise habits), despite being instructed to maintain their normal routine, because they know they are being observed or studied (Adair, 1984). For example, a period of exercise training can lead to unintentional positive changes in dietary patterns among adults (Joo et al., 2019). However, this limitation was minimized in *Chapter 7* through the inclusion of a control group received a placebo supplement.

8.4 Future Directions

The findings from the research program have raised several additional questions that warrant future investigation:

(1) Does exercise training affect the *in-vivo* response to DAPT in patients with acute coronary syndrome? In *Chapter 4*, we performed exercise training in healthy postmenopausal women and accordingly utilized *in-vitro* DAPT treatment. To provide real-world applicability, this training intervention would need to be

repeated in postmenopausal women with acute coronary syndrome currently taking DAPT.

(2) What are the mechanisms underpinning the *exercise timing hypothesis*? In *Chapter 6*, we showed divergent vascular responses to exercise training between recently and late postmenopausal women. However, we did not unearth the specific mechanisms underpinning these results. We believe that endothelial cell function may be a prime driver of the divergent vascular responses between recently and late postmenopausal women. Accordingly, we are currently investigating the effect of 8 weeks of high-intensity exercise training on isolated endothelial cells from skeletal muscle biopsies from recently and late postmenopausal women. We will unearth the differences in endothelial cell function between the two cohorts by evaluating: mitochondrial respiration, proliferation, migration, histochemical analysis for skeletal muscle capillarization, as well as protein content and gene expression.

(3) Are the differences in vascular adaptations to exercise training observed between recently and late postmenopausal women temporal or absolute? It is critical to understand whether late postmenopausal women simply take longer to achieve the same magnitude of adaptation to exercise training or if they lose the potential to recover vascular health. Although logistically complicated and demanding, a longer exercise training intervention (e.g., 16 weeks) with serial measurements (e.g., every 4 weeks) is needed to answer this question.

(4) Are the *exercise timing hypothesis* results reproducible worldwide? It is interesting to consider whether the degree of age- and menopause-related vascular damage is lower in Danish postmenopausal women compared to women in other countries around the globe. Therefore, it is possible that the window for optimal adaptations to an exercise intervention is shorter, and exercise interventions needs to be initiated earlier in these countries.

(5) Does combined exercise training and supplementation with fermented red clover extract have a synergistic effect on vascular health in recently postmenopausal women? This dissertation highlights the beneficial effects of both exercise training (*Chapters 4, 5, and 6*) and supplementation with fermented red clover extract (*Chapter 7*) in postmenopausal women. However, the combined effect of these interventions has not been evaluated. It is possible that the combination of supplementation with fermented red clover extract and exercise training could maximize the estrogen receptor and ERR interactions as well as antioxidant potential to promote vascular health in postmenopausal women.

8.5 References

Adair, J.G., 1984. The Hawthorne effect: A reconsideration of the methodological artifact. Journal of Applied Psychology 69, 334–345. https://doi.org/10.1037/0021-9010.69.2.334

Alfarisi, H.A.H., Mohamed, Z.B.H., Ibrahim, M.B., 2020. Basic pathogenic mechanisms of atherosclerosis. Egyptian Journal of Basic and Applied Sciences 7, 116–125. https://doi.org/10.1080/2314808X.2020.1769913

Alkarithi, G., Duval, C., Shi, Y., Macrae, F.L., Ariëns, R.A.S., 2021. Thrombus Structural Composition in Cardiovascular Disease. Arteriosclerosis, Thrombosis, and Vascular Biology 41, 2370–2383. https://doi.org/10.1161/ATVBAHA.120.315754

Badeau, M., Adlercreutz, H., Kaihovaara, P., Tikkanen, M.J., 2005. Estrogen A-ring structure and antioxidative effect on lipoproteins. The Journal of Steroid Biochemistry and Molecular Biology 96, 271–278. https://doi.org/10.1016/j.jsbmb.2005.04.034

Balsano, F., Rizzon, P., Violi, F., Scrutinio, D., Cimminiello, C., Aguglia, F., Pasotti, C., Rudelli, G., 1990. Antiplatelet treatment with ticlopidine in unstable angina. A controlled multicenter clinical trial. The Studio della Ticlopidina nell'Angina Instabile Group. Circulation 82, 17–26. https://doi.org/10.1161/01.cir.82.1.17

Barton, M., Meyer, M.R., 2009. Postmenopausal hypertension: mechanisms and therapy. Hypertension 54, 11–18. https://doi.org/10.1161/HYPERTENSIONAHA.108.120022

Batty, P., Smith, J.G., 2010. Haemostasis. Surgery (Oxford), Emergency Surgery 28, 530–535. https://doi.org/10.1016/j.mpsur.2010.08.008

Bedell, S., Nachtigall, M., Naftolin, F., 2014. The pros and cons of plant estrogens for menopause. The Journal of Steroid Biochemistry and Molecular Biology 139, 225–236. https://doi.org/10.1016/j.jsbmb.2012.12.004

Bejma, J., Ji, L.L., 1999. Aging and acute exercise enhance free radical generation in rat skeletal muscle. J Appl Physiol (1985) 87, 465–470. https://doi.org/10.1152/jappl.1999.87.1.465

Bergstrom, J., 1975. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scand J Clin Lab Invest 35, 609–616.

Bertrand, M.E., Rupprecht, H.J., Urban, P., Gershlick, A.H., CLASSICS Investigators, 2000. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting : the clopidogrel aspirin stent international cooperative study (CLASSICS). Circulation 102, 624–629. https://doi.org/10.1161/01.cir.102.6.624

Bittner, V., 2009. Menopause, Age, and Cardiovascular Risk. Journal of the American College of Cardiology 54, 2374–2375. https://doi.org/10.1016/j.jacc.2009.10.008

Bossavy, J.-P., Thalamas, C., Sagnard, L., Barret, A., Sakariassen, K., Boneu, B., Cadroy, Y., 1998. A Double-Blind Randomized Comparison of Combined Aspirin and Ticlopidine Therapy Versus Aspirin or Ticlopidine Alone on Experimental Arterial

Thrombogenesis in Humans. Blood 92, 1518–1525. https://doi.org/10.1182/blood.V92.5.1518
Bouchard, C., An, P., Rice, T., Skinner, J.S., Wilmore, J.H., Gagnon, J., Pérusse, L., Leon, A.S., Rao, D.C., 1999. Familial aggregation ofV˙o 2 max response to exercise training: results from the HERITAGE Family Study. Journal of Applied Physiology 87, 1003–1008. https://doi.org/10.1152/jappl.1999.87.3.1003
Bourgeois, F.T., Orenstein, L., Ballakur, S., Mandl, K.D., Ioannidis, J.P.A., 2017. Exclusion of Elderly People from Randomized Clinical Trials of Drugs for Ischemic Heart Disease. J Am Geriatr Soc 65, 2354–2361. https://doi.org/10.1111/jgs.14833
Brass, L.F., 2003. Thrombin and Platelet Activation. Chest 124, 18S-25S. https://doi.org/10.1378/chest.124.3_suppl.18S
Breet, N.J., Sluman, M.A., van Berkel, M.A.J.P.J., van Werkum, J.W., Bouman, H.J., Harmsze, A.M., Kelder, J.C., Zijlstra, F., Hackeng, C.M., ten Berg, J.M., 2011. Effect of gender difference on platelet reactivity. Neth Heart J 19, 451–457. https://doi.org/10.1007/s12471-011-0189-y
Briasoulis, A., Telila, T., Palla, M., Siasos, G., Tousoulis, D., 2016. P2Y12 Receptor Antagonists: Which One to Choose? A Systematic Review and Meta-Analysis. Curr Pharm Des 22, 4568–4576. https://doi.org/10.2174/1381612822666160608114424
Brown, R.G., Davidson, L.A.G., Mckeown, T., Whitfield, A.G.W., 1957. CORONARY-ARTERY DISEASE: INFLUENCES AFFECTING ITS INCIDENCE IN MALES IN THE SEVENTH DECADE. The Lancet, Originally published as Volume 2, Issue 7005 270, 1073–1077. https://doi.org/10.1016/S0140-6736(57)90113-7
Brownley, K.A., Hinderliter, A.L., West, S.G., Grewen, K.M., Steege, J.F., Girdler, S.S., Light, K.C., 2004. Cardiovascular effects of 6 months of hormone replacement therapy versus placebo: differences associated with years since menopause. American Journal of Obstetrics and Gynecology 190, 1052–1058. https://doi.org/10.1016/j.ajog.2003.09.045
Brzozowska, M., Lewiński, A., 2020. Changes of androgens levels in menopausal women. Prz Menopauzalny 19, 151–154. https://doi.org/10.5114/pm.2020.101941
Burger, H.G., 2002. Androgen production in women. Fertil Steril 77 Suppl 4, S3-5. https://doi.org/10.1016/s0015-0282(02)02985-0
Cauley, J.A., Robbins, J., Chen, Z., Cummings, S.R., Jackson, R.D., LaCroix, A.Z., LeBoff, M., Lewis, C.E., McGowan, J., Neuner, J., Pettinger, M., Stefanick, M.L., Wactawski-Wende, J., Watts, N.B., for the Women's Health Initiative Investigators, 2003. Effects of Estrogen Plus Progestin on Risk of Fracture and Bone Mineral DensityThe Women's Health Initiative Randomized Trial. JAMA 290, 1729–1738. https://doi.org/10.1001/jama.290.13.1729
Celermajer, D.S., Sorensen, K.E., Spiegelhalter, D.J., Georgakopoulos, D., Robinson, J., Deanfield, J.E., 1994. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol 24, 471–476. https://doi.org/10.1016/0735-1097(94)90305-0
Chan, M.V., Knowles, R.B.M., Lundberg, M.H., Tucker, A.T., Mohamed, N.A., Kirkby, N.S., Armstrong, P.C.J., Mitchell, J.A., Warner, T.D., 2016. P2Y12 receptor blockade

synergizes strongly with nitric oxide and prostacyclin to inhibit platelet activation. Br J Clin Pharmacol 81, 621–633. https://doi.org/10.1111/bcp.12826

Chapin, J.C., Hajjar, K.A., 2015. Fibrinolysis and the control of blood coagulation. Blood Rev 29, 17–24. https://doi.org/10.1016/j.blre.2014.09.003

Chlebowski, R.T., Wactawski-Wende, J., Ritenbaugh, C., Hubbell, F.A., Ascensao, J., Rodabough, R.J., Rosenberg, C.A., Taylor, V.M., Harris, R., Chen, C., Adams-Campbell, L.L., White, E., 2004. Estrogen plus Progestin and Colorectal Cancer in Postmenopausal Women. New England Journal of Medicine 350, 991–1004. https://doi.org/10.1056/NEJMoa032071

Cho, L., Mukherjee, D., 2005. Hormone replacement therapy and secondary cardiovascular prevention: a meta-analysis of randomized trials. Cardiology 104, 143–147. https://doi.org/10.1159/000087635

Chrzanowski-Smith, O.J., Piatrikova, E., Betts, J.A., Williams, S., Gonzalez, J.T., 2020. Variability in exercise physiology: Can capturing intra-individual variation help better understand true inter-individual responses? European Journal of Sport Science 20, 452–460. https://doi.org/10.1080/17461391.2019.1655100

Ciana, P., Raviscioni, M., Mussi, P., Vegeto, E., Que, I., Parker, M.G., Lowik, C., Maggi, A., 2003. In vivo imaging of transcriptionally active estrogen receptors. Nat Med 9, 82–86. https://doi.org/10.1038/nm809

Colacurci, N., Chiàntera, A., Fornaro, F., de Novellis, V., Manzella, D., Arciello, A., Chiàntera, V., Improta, L., Paolisso, G., 2005. Effects of soy isoflavones on endothelial function in healthy postmenopausal women. Menopause 12, 299–307. https://doi.org/10.1097/01.GME.0000147017.23173.5B

Collet, J.-P., Thiele, H., Barbato, E., Barthélémy, O., Bauersachs, J., Bhatt, D.L., Dendale, P., Dorobantu, M., Edvardsen, T., Folliguet, T., Gale, C.P., Gilard, M., Jobs, A., Jüni, P., Lambrinou, E., Lewis, B.S., Mehilli, J., Meliga, E., Merkely, B., Mueller, C., Roffi, M., Rutten, F.H., Sibbing, D., Siontis, G.C.M., ESC Scientific Document Group, Kastrati, A., Mamas, M.A., Aboyans, V., Angiolillo, D.J., Bueno, H., Bugiardini, R., Byrne, R.A., Castelletti, S., Chieffo, A., Cornelissen, V., Crea, F., Delgado, V., Drexel, H., Gierlotka, M., Halvorsen, S., Haugaa, K.H., Jankowska, E.A., Katus, H.A., Kinnaird, T., Kluin, J., Kunadian, V., Landmesser, U., Leclercq, C., Lettino, M., Meinila, L., Mylotte, D., Ndrepepa, G., Omerovic, E., Pedretti, R.F.E., Petersen, S.E., Petronio, A.S., Pontone, G., Popescu, B.A., Potpara, T., Ray, K.K., Luciano, F., Richter, D.J., Shlyakhto, E., Simpson, I.A., Sousa-Uva, M., Storey, R.F., Touyz, R.M., Valgimigli, M., Vranckx, P., Yeh, R.W., Barbato, E., Barthélémy, O., Bauersachs, J., Bhatt, D.L., Dendale, P., Dorobantu, M., Edvardsen, T., Folliguet, T., Gale, C.P., Gilard, M., Jobs, A., Jüni, P., Lambrinou, E., Lewis, B.S., Mehilli, J., Meliga, E., Merkely, B., Mueller, C., Roffi, M., Rutten, F.H., Sibbing, D., Siontis, G.C.M., 2021. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. European Heart Journal 42, 1289–1367. https://doi.org/10.1093/eurheartj/ehaa575

Costello, J.T., Bieuzen, F., Bleakley, C.M., 2014. Where are all the female participants in Sports and Exercise Medicine research? European Journal of Sport Science 14, 847–851. https://doi.org/10.1080/17461391.2014.911354

Couse, J.F., Lindzey, J., Grandien, K., Gustafsson, J.A., Korach, K.S., 1997. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 138, 4613–4621. https://doi.org/10.1210/endo.138.11.5496

Craige, S.M., Kröller-Schön, S., Li, C., Kant, S., Cai, S., Chen, K., Contractor, M.M., Pei, Y., Schulz, E., Keaney, J.F., 2016. PGC-1α dictates endothelial function through regulation of eNOS expression. Sci Rep 6, 38210. https://doi.org/10.1038/srep38210

Curtis, D.J., Brown, M.R., Hawkins, K., Evans, P.A., Lawrence, M.J., Rees, P., Williams, P.R., 2011. Rheometrical and molecular dynamics simulation studies of incipient clot formation in fibrin-thrombin gels: An activation limited aggregation approach. Journal of Non-Newtonian Fluid Mechanics, Papers Presented at the University of Wales Institute of Non-Newtonian Fluid Mechanics Meeting on Rheometry, 29-31 March 2010, Lake Vyrnwy, Wales 166, 932–938. https://doi.org/10.1016/j.jnnfm.2011.04.016

Curtis, D.J., Williams, P.R., Badiei, N., Campbell, A.I., Hawkins, K., Evans, P.A., Brown, M.R., 2013. A study of microstructural templating in fibrin–thrombin gel networks by spectral and viscoelastic analysis. Soft Matter 9, 4883–4889. https://doi.org/10.1039/C3SM50263E

Danforth, D.R., Arbogast, L.K., Mroueh, J., Kim, M.H., Kennard, E.A., Seifer, D.B., Friedman, C.I., 1998. Dimeric inhibin: a direct marker of ovarian aging. Fertil Steril 70, 119–123. https://doi.org/10.1016/s0015-0282(98)00127-7

Dastjerdi, M.S., Emami, T., Najafian, A., Amini, M., 2006. Mean platelet volume measurement, EDTA or citrate? Hematology 11, 317–319. https://doi.org/10.1080/10245330600954163

Davie, E.W., Ratnoff, O.D., 1964. Waterfall Sequence for Intrinsic Blood Clotting. Science 145, 1310–1312. https://doi.org/10.1126/science.145.3638.1310

Davies, N.A., Llwyd, O., Brugniaux, J.V., Davies, G.R., Marley, C.J., Hodson, D., Lawrence, M.J., D'Silva, L.A., Morris, R.H.K., Hawkins, K., Williams, P.R., Bailey, D.M., Evans, P.A., 2016. Effects of exercise intensity on clot microstructure and mechanical properties in healthy individuals. Thrombosis Research 143, 130–136. https://doi.org/10.1016/j.thromres.2016.05.018

DeSouza, C.A., Shapiro, L.F., Clevenger, C.M., Dinenno, F.A., Monahan, K.D., Tanaka, H., Seals, D.R., 2000. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 102, 1351–1357. https://doi.org/10.1161/01.cir.102.12.1351

Dhalla, N.S., Temsah, R.M., Netticadan, T., 2000. Role of oxidative stress in cardiovascular diseases. J Hypertens 18, 655–673. https://doi.org/10.1097/00004872-200018060-00002

Dobesh, P.P., Oestreich, J.H., 2014. Ticagrelor: Pharmacokinetics, Pharmacodynamics, Clinical Efficacy, and Safety. Pharmacotherapy 34, 1077–1090. https://doi.org/10.1002/phar.1477

Dorsam, R.T., Kunapuli, S.P., 2004. Central role of the $P2Y_{12}$ receptor in platelet activation. J Clin Invest 113, 340–345. https://doi.org/10.1172/JCI20986

Egelund, J., Jørgensen, P.G., Mandrup, C.M., Fritz-Hansen, T., Stallknecht, B., Bangsbo, J., Nyberg, M., Hellsten, Y., 2017. Cardiac Adaptations to High-Intensity Aerobic Training in Premenopausal and Recent Postmenopausal Women: The Copenhagen Women Study. J Am Heart Assoc 6, e005469. https://doi.org/10.1161/JAHA.117.005469

El Khoudary, S.R., Aggarwal, B., Beckie, T.M., Hodis, H.N., Johnson, A.E., Langer, R.D., Limacher, M.C., Manson, J.E., Stefanick, M.L., Allison, M.A., null, null, 2020. Menopause Transition and Cardiovascular Disease Risk: Implications for Timing of Early Prevention: A Scientific Statement From the American Heart Association. Circulation 142, e506–e532. https://doi.org/10.1161/CIR.0000000000000912

Elmariah, S., Mauri, L., Doros, G., Galper, B.Z., O'Neill, K.E., Steg, P.G., Kereiakes, D.J., Yeh, R.W., 2015. Extended duration dual antiplatelet therapy and mortality: a systematic review and meta-analysis. The Lancet 385, 792–798. https://doi.org/10.1016/S0140-6736(14)62052-3

Engbers, M.J., Van Hylckama Vlieg, A., Rosendaal, F.R., 2010. Venous thrombosis in the elderly: incidence, risk factors and risk groups. Journal of Thrombosis and Haemostasis 8, 2105–2112. https://doi.org/10.1111/j.1538-7836.2010.03986.x

Finkelstein, J.S., Brockwell, S.E., Mehta, V., Greendale, G.A., Sowers, M.R., Ettinger, B., Lo, J.C., Johnston, J.M., Cauley, J.A., Danielson, M.E., Neer, R.M., 2008. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J Clin Endocrinol Metab 93, 861–868. https://doi.org/10.1210/jc.2007-1876

FitzGerald, G.A., Oates, J.A., Hawiger, J., Maas, R.L., Roberts, L.J., Lawson, J.A., Brash, A.R., 1983. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest 71, 676–688. https://doi.org/10.1172/JCI110814

Fleming, I., 2010. Molecular mechanisms underlying the activation of eNOS. Pflugers Arch 459, 793–806. https://doi.org/10.1007/s00424-009-0767-7

Fogle, R.H., Stanczyk, F.Z., Zhang, X., Paulson, R.J., 2007. Ovarian androgen production in postmenopausal women. J Clin Endocrinol Metab 92, 3040–3043. https://doi.org/10.1210/jc.2007-0581

Förstermann, U., Münzel, T., 2006. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113, 1708–1714. https://doi.org/10.1161/CIRCULATIONAHA.105.602532

Fournier, A., Mesrine, S., Dossus, L., Boutron-Ruault, M.-C., Clavel-Chapelon, F., Chabbert-Buffet, N., 2014. Risk of breast cancer after stopping menopausal hormone therapy in the E3N cohort. Breast Cancer Res Treat 145, 535–543. https://doi.org/10.1007/s10549-014-2934-6

Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri, M., Salvioli, S., 2018. The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. Frontiers in Medicine 5.

Francis, A.A., Pierce, G.N., 2011. An integrated approach for the mechanisms responsible for atherosclerotic plaque regression. Exp Clin Cardiol 16, 77–86.

Fredette, N.C., Meyer, M.R., Prossnitz, E.R., 2018. Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. J Steroid Biochem Mol Biol 176, 65–72. https://doi.org/10.1016/j.jsbmb.2017.05.006

Fuentes, N., Silveyra, P., 2019. Estrogen receptor signaling mechanisms. Advances in protein chemistry and structural biology 116, 135. https://doi.org/10.1016/bs.apcsb.2019.01.001

Fuller, R., Chavez, B., 2012. Ticagrelor (Brilinta), an Antiplatelet Drug for Acute Coronary Syndrome. P T 37, 562–568.

Gale, A.J., 2011. Current Understanding of Hemostasis. Toxicol Pathol 39, 273–280. https://doi.org/10.1177/0192623310389474

García-Martínez, M.C., Hermenegildo, C., Tarín, J.J., Cano, A., 2003. Phytoestrogens increase the capacity of serum to stimulate prostacyclin release in human endothelial cells. Acta Obstet Gynecol Scand 82, 705–710. https://doi.org/10.1080/j.1600-0412.2003.00178.x

Gent, M., Blakely, J.A., Easton, J.D., Ellis, D.J., Hachinski, V.C., Harbison, J.W., Panak, E., Roberts, R.S., Sicurella, J., Turpie, A.G., 1989. The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. Lancet 1, 1215–1220. https://doi.org/10.1016/s0140-6736(89)92327-1

Gliemann, L., Hellsten, Y., 2019. The exercise timing hypothesis: can exercise training compensate for the reduction in blood vessel function after menopause if timed right? The Journal of Physiology 597, 4915–4925. https://doi.org/10.1113/JP277056

Gliemann, L., Rytter, N., Tamariz-Ellemann, A., Egelund, J., Brandt, N., Carter, H.H., Hellsten, Y., 2020. Lifelong Physical Activity Determines Vascular Function in Late Postmenopausal Women. Med Sci Sports Exerc 52, 627–636. https://doi.org/10.1249/MSS.0000000000002180

Goldman, J.A., 2004. The Women's Health Initiative 2004 - Review and Critique. MedGenMed 6, 65.

Government of Canada, 2021. Canadian Health Measures Survey: Activity monitor data, 2018-2019 [WWW Document]. URL https://www150.statcan.gc.ca/n1/daily-quotidien/210901/dq210901c-eng.htm (accessed 10.12.22).

Grady, D., Rubin, S.M., Petitti, D.B., Fox, C.S., Black, D., Ettinger, B., Ernster, V.L., Cummings, S.R., 1992. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 117, 1016–1037. https://doi.org/10.7326/0003-4819-117-12-1016

Green, D.J., Maiorana, A., O'Driscoll, G., Taylor, R., 2004. Effect of exercise training on endothelium-derived nitric oxide function in humans. J Physiol 561, 1–25. https://doi.org/10.1113/jphysiol.2004.068197

Greendale, G.A., Reboussin, B.A., Hogan, P., Barnabei, V.M., Shumaker, S., Johnson, S., Barrett-Connor, E., 1998. Symptom relief and side effects of postmenopausal hormones: results from the postmenopausal estrogen/progestin interventions trial. Obstetrics & Gynecology 92, 982–988. https://doi.org/10.1016/S0029-7844(98)00305-6

Gregorini, L., Marco, J., 1997. Ticlopidine and aspirin interactions. Heart 77, 11–12. https://doi.org/10.1136/hrt.77.1.11

Grindler, N.M., Santoro, N.F., 2015. Menopause and exercise. Menopause 22, 1351–1358. https://doi.org/10.1097/GME.0000000000000536

Grodstein, F., Stampfer, M.J., Colditz, G.A., Willett, W.C., Manson, J.E., Joffe, M., Rosner, B., Fuchs, C., Hankinson, S.E., Hunter, D.J., Hennekens, C.H., Speizer, F.E., 1997. Postmenopausal Hormone Therapy and Mortality. New England Journal of Medicine 336, 1769–1776. https://doi.org/10.1056/NEJM199706193362501

Hackley, B., Rousseau, M.E., 2004. Managing menopausal symptoms after the women's health initiative. Journal of Midwifery & Women's Health 49, 87–95. https://doi.org/10.1016/j.jmwh.2003.12.005

Hall, W.L., Vafeiadou, K., Hallund, J., Bügel, S., Koebnick, C., Reimann, M., Ferrari, M., Branca, F., Talbot, D., Dadd, T., Nilsson, M., Dahlman-Wright, K., Gustafsson, J.-A., Minihane, A.-M., Williams, C.M., 2005. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. Am J Clin Nutr 82, 1260–1268; quiz 1365–1366. https://doi.org/10.1093/ajcn/82.6.1260

Hamilos, M., Petousis, S., Parthenakis, F., 2018. Interaction between platelets and endothelium: from pathophysiology to new therapeutic options. Cardiovasc Diagn Ther 8, 568–580. https://doi.org/10.21037/cdt.2018.07.01

Hamilton, K.J., Hewitt, S.C., Arao, Y., Korach, K.S., 2017. Estrogen Hormone Biology. Curr Top Dev Biol 125, 109–146. https://doi.org/10.1016/bs.ctdb.2016.12.005

Hanby, H.A., Bao, J., Noh, J.-Y., Jarocha, D., Poncz, M., Weiss, M.J., Marks, M.S., 2017. Platelet dense granules begin to selectively accumulate mepacrine during proplatelet formation. Blood Advances 1, 1478–1490. https://doi.org/10.1182/bloodadvances.2017006726

Haney, A., Wild, R., 2007. Options for hormone therapy in women who have had a hysterectomy. Menopause (New York, N.Y.) 14. https://doi.org/10.1097/gme.0b013e31804154d5

Harlow, S.D., Mitchell, E.S., Crawford, S., Nan, B., Little, R., Taffe, J., ReSTAGE Collaboration, 2008. The ReSTAGE Collaboration: defining optimal bleeding criteria for onset of early menopausal transition. Fertil Steril 89, 129–140. https://doi.org/10.1016/j.fertnstert.2007.02.015

Harper-Harrison, G., Shanahan, M.M., 2022. Hormone Replacement Therapy, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Harridge, S.D.R., Lazarus, N.R., 2017. Physical Activity, Aging, and Physiological Function. Physiology (Bethesda) 32, 152–161. https://doi.org/10.1152/physiol.00029.2016

Harris, G.W., 1948. Electrical stimulation of the hypothalamus and the mechanism of neural control of the adenohypophysis. J Physiol 107, 418–429. https://doi.org/10.1113/jphysiol.1948.sp004286
Hass, W.K., Easton, J.D., Adams, H.P., Pryse-Phillips, W., Molony, B.A., Anderson, S., Kamm, B., 1989. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. Ticlopidine Aspirin Stroke Study Group. N Engl J Med 321, 501–507. https://doi.org/10.1056/NEJM198908243210804
Hawley, J.A., Burke, L.M., Phillips, S.M., Spriet, L.L., 2011. Nutritional modulation of training-induced skeletal muscle adaptations. J Appl Physiol (1985) 110, 834–845. https://doi.org/10.1152/japplphysiol.00949.2010
He, F., Li, J., Liu, Z., Chuang, C.-C., Yang, W., Zuo, L., 2016. Redox Mechanism of Reactive Oxygen Species in Exercise. Front Physiol 7, 486. https://doi.org/10.3389/fphys.2016.00486
Hecker, M., Ullrich, V., 1989. On the mechanism of prostacyclin and thromboxane A2 biosynthesis. J Biol Chem 264, 141–150.
Henderson, B.E., Paganini-Hill, A., Ross, R.K., 1991. Decreased Mortality in Users of Estrogen Replacement Therapy. Archives of Internal Medicine 151, 75–78. https://doi.org/10.1001/archinte.1991.00400010095012
Hiller, E., 2007. Basic Principles of Hemostasis, in: Munker, R., Hiller, E., Glass, J., Paquette, R. (Eds.), Modern Hematology: Biology and Clinical Management, Contemporary Hematology. Humana Press, Totowa, NJ, pp. 327–345. https://doi.org/10.1007/978-1-59745-149-9_19
Hinchliff, S., 2019. Menopause, in: Gu, D., Dupre, M.E. (Eds.), Encyclopedia of Gerontology and Population Aging. Springer International Publishing, Cham, pp. 1–5. https://doi.org/10.1007/978-3-319-69892-2_161-1
Hodis, H.N., Mack, W.J., Henderson, V.W., Shoupe, D., Budoff, M.J., Hwang-Levine, J., Li, Y., Feng, M., Dustin, L., Kono, N., Stanczyk, F.Z., Selzer, R.H., Azen, S.P., 2016. Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol. N Engl J Med 374, 1221–1231. https://doi.org/10.1056/NEJMoa1505241
Hoffman, B.B., Michel, T., Brenneman, T.B., Lefkowitz, R.J., 1982. Interactions of agonists with platelet alpha 2-adrenergic receptors. Endocrinology 110, 926–932. https://doi.org/10.1210/endo-110-3-926
Hoier, B., Nordsborg, N., Andersen, S., Jensen, L., Nybo, L., Bangsbo, J., Hellsten, Y., 2012. Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. The Journal of Physiology 590, 595–606. https://doi.org/10.1113/jphysiol.2011.216135
Hoier, B., Olsen, L.N., Leinum, M., Jørgensen, T.S., Carter, H.H., Hellsten, Y., Bangsbo, J., 2021. Aerobic High-Intensity Exercise Training Improves Cardiovascular Health in Older Post-menopausal Women. Front Aging 2, 667519. https://doi.org/10.3389/fragi.2021.667519
Holder, S.M., Brislane, Á., Dawson, E.A., Hopkins, N.D., Hopman, M.T.E., Cable, N.T., Jones, H., Schreuder, T.H.A., Sprung, V.S., Naylor, L., Maiorana, A., Thompson, A., Thijssen, D.H.J., Green, D.J., 2019. Relationship Between Endothelial Function

and the Eliciting Shear Stress Stimulus in Women: Changes Across the Lifespan Differ to Men. J Am Heart Assoc 8, e010994. https://doi.org/10.1161/JAHA.118.010994

Horn-Ross, P.L., John, E.M., Canchola, A.J., Stewart, S.L., Lee, M.M., 2003. Phytoestrogen intake and endometrial cancer risk. J Natl Cancer Inst 95, 1158–1164. https://doi.org/10.1093/jnci/djg015

Horstman, A.M., Dillon, E.L., Urban, R.J., Sheffield-Moore, M., 2012. The Role of Androgens and Estrogens on Healthy Aging and Longevity. J Gerontol A Biol Sci Med Sci 67, 1140–1152. https://doi.org/10.1093/gerona/gls068

Howes, J.B., Tran, D., Brillante, D., Howes, L.G., 2003. Effects of dietary supplementation with isoflavones from red clover on ambulatory blood pressure and endothelial function in postmenopausal type 2 diabetes. Diabetes Obes Metab 5, 325–332. https://doi.org/10.1046/j.1463-1326.2003.00282.x

Hsia, J., Langer, R.D., Manson, J.E., Kuller, L., Johnson, K.C., Hendrix, S.L., Pettinger, M., Heckbert, S.R., Greep, N., Crawford, S., Eaton, C.B., Kostis, J.B., Caralis, P., Prentice, R., Women's Health Initiative Investigators, 2006. Conjugated equine estrogens and coronary heart disease: the Women's Health Initiative. Arch Intern Med 166, 357–365. https://doi.org/10.1001/archinte.166.3.357

Huveneers, S., Daemen, M.J.A.P., Hordijk, P.L., 2015. Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. Circ Res 116, 895–908. https://doi.org/10.1161/CIRCRESAHA.116.305720

Hyde, E.T., Whitfield, G.P., Omura, J.D., Fulton, J.E., Carlson, S.A., 2021. Trends in Meeting the Physical Activity Guidelines: Muscle-Strengthening Alone and Combined With Aerobic Activity, United States, 1998–2018. Journal of Physical Activity and Health 18, S37–S44. https://doi.org/10.1123/jpah.2021-0077

Iqbal, J., Zaidi, M., 2009. Understanding Estrogen Action during Menopause. Endocrinology 150, 3443–3445. https://doi.org/10.1210/en.2009-0449

Izquierdo, M., Häkkinen, K., Antón, A., Garrues, M., Ibañez, J., Ruesta, M., Gorostiaga, E.M., 2001. Maximal strength and power, endurance performance, and serum hormones in middle-aged and elderly men. Med Sci Sports Exerc 33, 1577–1587. https://doi.org/10.1097/00005768-200109000-00022

Izzo, C., Vitillo, P., Di Pietro, P., Visco, V., Strianese, A., Virtuoso, N., Ciccarelli, M., Galasso, G., Carrizzo, A., Vecchione, C., 2021. The Role of Oxidative Stress in Cardiovascular Aging and Cardiovascular Diseases. Life (Basel) 11, 60. https://doi.org/10.3390/life11010060

Jin, J., Daniel, J.L., Kunapuli, S.P., 1998. Molecular basis for ADP-induced platelet activation. II. The P2Y1 receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. J Biol Chem 273, 2030–2034. https://doi.org/10.1074/jbc.273.4.2030

Jones, S.P., Bolli, R., 2006. The ubiquitous role of nitric oxide in cardioprotection. Journal of Molecular and Cellular Cardiology 40, 16–23. https://doi.org/10.1016/j.yjmcc.2005.09.011

Joo, J., Williamson, S.A., Vazquez, A.I., Fernandez, J.R., Bray, M.S., 2019. The influence of 15-week exercise training on dietary patterns among young adults. Int J Obes 43, 1681–1690. https://doi.org/10.1038/s41366-018-0299-3

Joswig, M., Hach-Wunderle, V., von Holst, T., Nawroth, P.P., 2000. Postmenopausal hormone replacement therapy and the vascular wall: epidemiology and clinical studies. Vasa 29, 243–251. https://doi.org/10.1024/0301-1526.29.4.243

Kalhan, M., Singhania, K., Choudhary, P., Verma, S., Kaushal, P., Singh, T., 2020. Prevalence of Menopausal Symptoms and its Effect on Quality of Life among Rural Middle Aged Women (40–60 Years) of Haryana, India. Int J Appl Basic Med Res 10, 183–188. https://doi.org/10.4103/ijabmr.IJABMR_428_19

Kerlikowske, K., Miglioretti, D.L., Ballard-Barbash, R., Weaver, D.L., Buist, D.S.M., Barlow, W.E., Cutter, G., Geller, B.M., Yankaskas, B., Taplin, S.H., Carney, P.A., 2003. Prognostic Characteristics of Breast Cancer Among Postmenopausal Hormone Users in a Screened Population. JCO 21, 4314–4321. https://doi.org/10.1200/JCO.2003.05.151

Kim, M.R., Kim, H.J., Yu, S.H., Lee, B.S., Jeon, S.Y., Lee, J.J., Lee, Y.C., 2020. Combination of Red Clover and Hops Extract Improved Menopause Symptoms in an Ovariectomized Rat Model. Evid Based Complement Alternat Med 2020, 7941391. https://doi.org/10.1155/2020/7941391

Kim, Y., Lee, E., 2019. The association between elderly people's sedentary behaviors and their health-related quality of life: focusing on comparing the young-old and the old-old. Health and Quality of Life Outcomes 17, 131. https://doi.org/10.1186/s12955-019-1191-0

Kirkby, N.S., Lundberg, M.H., Chan, M.V., Vojnovic, I., Solomon, A.B., Emerson, M., Mitchell, J.A., Warner, T.D., 2013. Blockade of the purinergic P2Y12 receptor greatly increases the platelet inhibitory actions of nitric oxide. Proc Natl Acad Sci U S A 110, 15782–15787. https://doi.org/10.1073/pnas.1218880110

Koenig, A., Buskiewicz, I., Huber, S.A., 2017. Age-Associated Changes in Estrogen Receptor Ratios Correlate with Increased Female Susceptibility to Coxsackievirus B3-Induced Myocarditis. Front Immunol 8, 1585. https://doi.org/10.3389/fimmu.2017.01585

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.A., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870. https://doi.org/10.1210/endo.138.3.4979

Lablanche, J.M., McFadden, E.P., Bonnet, J.L., Grollier, G., Danchin, N., Bedossa, M., Leclercq, C., Vahanian, A., Bauters, C., Van Belle, E., Bertrand, M.E., 1996. Combined antiplatelet therapy with ticlopidine and aspirin. A simplified approach to intracoronary stent management. Eur Heart J 17, 1373–1380. https://doi.org/10.1093/oxfordjournals.eurheartj.a015072

Laganière, J., Tremblay, G.B., Dufour, C.R., Giroux, S., Rousseau, F., Giguère, V., 2004. A polymorphic autoregulatory hormone response element in the human estrogen-related receptor alpha (ERRalpha) promoter dictates peroxisome proliferator-

activated receptor gamma coactivator-1alpha control of ERRalpha expression. J Biol Chem 279, 18504–18510. https://doi.org/10.1074/jbc.M313543200
Lawrence, M.J., Davies, G., Nyberg, M., Whitley, J., Evans, V., Williams, R., Hellsten, Y., Evans, P.A., 2018. The effect of tyramine infusion and exercise on blood flow, coagulation and clot microstructure in healthy individuals. Thrombosis Research 170, 32–37. https://doi.org/10.1016/j.thromres.2018.07.025
Lawrence, M.J., Kumar, S., Hawkins, K., Boden, S., Rutt, H., Mills, G., Sabra, A., Morris, R.H.K., Davidson, S.J., Badiei, N., Brown, M.R., Williams, P.R., Evans, P.A., 2014. A new structural biomarker that quantifies and predicts changes in clot strength and quality in a model of progressive haemodilution. Thrombosis Research 134, 488–494. https://doi.org/10.1016/j.thromres.2014.05.039
Lazarus, N.R., Harridge, S.D.R., 2017. Declining performance of master athletes: silhouettes of the trajectory of healthy human ageing? The Journal of Physiology 595, 2941–2948. https://doi.org/10.1113/JP272443
Le Blanc, J., Lordkipanidzé, M., 2019. Platelet Function in Aging. Front Cardiovasc Med 6, 109. https://doi.org/10.3389/fcvm.2019.00109
Lebon, J., Riesco, E., Tessier, D., Dionne, I.J., 2014. Additive effects of isoflavones and exercise training on inflammatory cytokines and body composition in overweight and obese postmenopausal women: a randomized controlled trial. Menopause 21, 869–875. https://doi.org/10.1097/GME.0000000000000177
Lecut, C., Schoolmeester, A., Kuijpers, M.J.E., Broers, J.L.V., van Zandvoort, M.A.M.J., Vanhoorelbeke, K., Deckmyn, H., Jandrot-Perrus, M., Heemskerk, J.W.M., 2004. Principal role of glycoprotein VI in alpha2beta1 and alphaIIbbeta3 activation during collagen-induced thrombus formation. Arterioscler Thromb Vasc Biol 24, 1727–1733. https://doi.org/10.1161/01.ATV.0000137974.85068.93
Lee, H.-L., Kim, J.-T., Lee, J.S., Park, M.-S., Choi, K.-H., Cho, K.-H., Kim, B.J., Park, J.-M., Kang, K., Lee, S.J., Kim, J.G., Cha, J.-K., Kim, D.-H., Park, T.H., Park, S.-S., Lee, K.B., Lee, Jun, Hong, K.-S., Cho, Y.-J., Park, H.-K., Lee, B.-C., Yu, K.-H., Sun Oh, M., Kim, D.-E., Ryu, W.-S., Choi, J.C., Kwon, J.-H., Kim, W.-J., Shin, D.-I., Sohn, S.I., Hong, J.-H., Lee, Juneyoung, Bae, H.-J., 2020. Comparative Effectiveness of Dual Antiplatelet Therapy With Aspirin and Clopidogrel Versus Aspirin Monotherapy in Mild-to-Moderate Acute Ischemic Stroke According to the Risk of Recurrent Stroke. Circulation: Cardiovascular Quality and Outcomes 13, e006474. https://doi.org/10.1161/CIRCOUTCOMES.119.006474
Lemežienė, N., Padarauskas, A., Butkutė, B., Cesevičienė, J., Taujenis, L., Norkevičienė, E., Mikaliūnienė, J., 2015. The concentration of isoflavones in red clover (Trifolium pratense L.) at flowering stage. Žemdirbystė (Agriculture) 102, 443–448.
Levick, J.R., 1991. Chapter 1 - Overview of the cardiovascular system, in: Levick, J.R. (Ed.), An Introduction to Cardiovascular Physiology. Butterworth-Heinemann, pp. 1–12. https://doi.org/10.1016/B978-0-7506-1028-5.50004-4
Levin, E.R., 2009. Plasma Membrane Estrogen Receptors. Trends Endocrinol Metab 20, 477–482. https://doi.org/10.1016/j.tem.2009.06.009

Li, B., Wang, J.H.-C., 2011. Fibroblasts and Myofibroblasts in Wound Healing: Force Generation and Measurement. J Tissue Viability 20, 108–120. https://doi.org/10.1016/j.jtv.2009.11.004
Li, C.I., Malone, K.E., Porter, P.L., Weiss, N.S., Tang, M.-T.C., Cushing-Haugen, K.L., Daling, J.R., 2003. Relationship Between Long Durations and Different Regimens of Hormone Therapy and Risk of Breast Cancer. JAMA 289, 3254–3263. https://doi.org/10.1001/jama.289.24.3254
Li, S.-H., Liu, X.-X., Bai, Y.-Y., Wang, X.-J., Sun, K., Chen, J.-Z., Hui, R.-T., 2010. Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. Am J Clin Nutr 91, 480–486. https://doi.org/10.3945/ajcn.2009.28203
Li, T.Y., Rana, J.S., Manson, J.E., Willett, W.C., Stampfer, M.J., Colditz, G.A., Rexrode, K.M., Hu, F.B., 2006. Obesity as Compared With Physical Activity in Predicting Risk of Coronary Heart Disease in Women. Circulation 113, 499–506. https://doi.org/10.1161/CIRCULATIONAHA.105.574087
Li, Z., Delaney, M.K., O'Brien, K.A., Du, X., 2010. Signaling during platelet adhesion and activation. Arterioscler Thromb Vasc Biol 30, 2341–2349. https://doi.org/10.1161/ATVBAHA.110.207522
Lievertz, R.W., 1987. Pharmacology and pharmacokinetics of estrogens. American Journal of Obstetrics and Gynecology 156, 1289–1293. https://doi.org/10.1016/0002-9378(87)90166-9
Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. Clin Interv Aging 13, 757–772. https://doi.org/10.2147/CIA.S158513
Liu, D., Zhang, Z., Gladwell, W., Teng, C.T., 2003. Estrogen Stimulates Estrogen-Related Receptor α Gene Expression through Conserved Hormone Response Elements. Endocrinology 144, 4894–4904. https://doi.org/10.1210/en.2003-0432
Liu, Y., Ma, L., Yang, X., Bie, J., Li, D., Sun, C., Zhang, J., Meng, Y., Lin, J., 2019. Menopausal Hormone Replacement Therapy and the Risk of Ovarian Cancer: A Meta-Analysis. Frontiers in Endocrinology 10.
Lundberg Slingsby, M.H., Gliemann, L., Thrane, M., Rytter, N., Egelund, J., Chan, M.V., Armstrong, P.C., Warner, T.D., Hellsten, Y., 2018. Platelet responses to pharmacological and physiological interventions in middle-aged men with different habitual physical activity levels. Acta Physiol 223, e13028. https://doi.org/10.1111/apha.13028
Lundberg Slingsby, M.H., Nyberg, M., Egelund, J., Mandrup, C.M., Frikke-Schmidt, R., Kirkby, N.S., Hellsten, Y., 2017. Aerobic exercise training lowers platelet reactivity and improves platelet sensitivity to prostacyclin in pre- and postmenopausal women. J Thromb Haemost 15, 2419–2431. https://doi.org/10.1111/jth.13866
Macfarlane, R.G., 1964. An Enzyme Cascade in the Blood Clotting Mechanism, and its Function as a Biochemical Amplifier. Nature 202, 498–499. https://doi.org/10.1038/202498a0
Mackman, N., 2009. The Role of Tissue Factor and Factor VIIa in Hemostasis. Anesth Analg 108, 1447–1452. https://doi.org/10.1213/ane.0b013e31819bceb1

Maessen, M.F.H., Verbeek, A.L.M., Bakker, E.A., Thompson, P.D., Hopman, M.T.E., Eijsvogels, T.M.H., 2016. Lifelong Exercise Patterns and Cardiovascular Health. Mayo Clinic Proceedings 91, 745–754. https://doi.org/10.1016/j.mayocp.2016.02.028

Magnette, A., Chatelain, M., Chatelain, B., Ten Cate, H., Mullier, F., 2016. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. Thromb J 14, 49. https://doi.org/10.1186/s12959-016-0123-z

Mainini, G., Torella, M., Di Donna, M.C., Esposito, E., Ercolano, S., Correa, R., Cucinella, G., Stradella, L., Luisi, A., Basso, A., Cerreto, F.V., Cicatiello, R., Matteo, M., De Franciscis, P., 2013. Nonhormonal management of postmenopausal women: effects of a red clover based isoflavones supplementation on climacteric syndrome and cardiovascular risk serum profile. Clin Exp Obstet Gynecol 40, 337–341.

Mallidi, J., Lata, K., 2019. Role of Gender in Dual Antiplatelet Therapy After Acute Coronary Syndrome. Curr Atheroscler Rep 21, 34. https://doi.org/10.1007/s11883-019-0795-5

Mandrup, C.M., Egelund, J., Nyberg, M., Slingsby, M.H.L., Andersen, C.B., Løgstrup, S., Bangsbo, J., Suetta, C., Stallknecht, B., Hellsten, Y., 2017. Effects of high-intensity training on cardiovascular risk factors in premenopausal and postmenopausal women. American Journal of Obstetrics & Gynecology 216, 384.e1-384.e11. https://doi.org/10.1016/j.ajog.2016.12.017

Mann, T.N., Lamberts, R.P., Lambert, M.I., 2014. High responders and low responders: factors associated with individual variation in response to standardized training. Sports Med 44, 1113–1124. https://doi.org/10.1007/s40279-014-0197-3

Manolis, A.S., Manolis, T.A., Papadimitriou, P., Koulouris, S., Melita, H., 2013. Combined antiplatelet therapy: still a sweeping combination in cardiology. Cardiovasc Hematol Agents Med Chem 11, 136–167. https://doi.org/10.2174/1871525711311020009

Mari, D., Ogliari, G., Castaldi, D., Vitale, G., Bollini, E.M., Lio, D., 2008. Hemostasis and ageing. Immun Ageing 5, 12. https://doi.org/10.1186/1742-4933-5-12

Markopoulos, M.C., Kassi, E., Alexandraki, K.I., Mastorakos, G., Kaltsas, G., 2015. Hyperandrogenism after menopause. Eur J Endocrinol 172, R79-91. https://doi.org/10.1530/EJE-14-0468

Marrocco, J., McEwen, B.S., 2016. Sex in the brain: hormones and sex differences. Dialogues Clin Neurosci 18, 373–383.

Marsden, J., 2002. The menopause, hormone replacement therapy and breast cancer. The Journal of Steroid Biochemistry and Molecular Biology, Proceedings of the 15th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology “Recent Advances in Steroid Biochemistry and Molecular Biology” 83, 123–132. https://doi.org/10.1016/S0960-0760(02)00258-3

Matthews, K.A., Crawford, S.L., Chae, C.U., Everson-Rose, S.A., Sowers, M.F., Sternfeld, B., Sutton-Tyrrell, K., 2009. Are Changes in Cardiovascular Disease Risk Factors in Midlife Women due to Chronological Aging or to the Menopausal Transition? J Am Coll Cardiol 54, 2366–2373. https://doi.org/10.1016/j.jacc.2009.10.009

Mauri, L., Kereiakes, D.J., Yeh, R.W., Driscoll-Shempp, P., Cutlip, D.E., Steg, P.G., Normand, S.-L.T., Braunwald, E., Wiviott, S.D., Cohen, D.J., Holmes, D.R., Krucoff, M.W., Hermiller, J., Dauerman, H.L., Simon, D.I., Kandzari, D.E., Garratt, K.N., Lee, D.P., Pow, T.K., Ver Lee, P., Rinaldi, M.J., Massaro, J.M., 2014. Twelve or 30 Months of Dual Antiplatelet Therapy after Drug-Eluting Stents. New England Journal of Medicine 371, 2155–2166. https://doi.org/10.1056/NEJMoa1409312

McDonnell, D.P., Norris, J.D., 2002. Connections and Regulation of the Human Estrogen Receptor. Science 296, 1642–1644. https://doi.org/10.1126/science.1071884

Mercado, C.P., Kilic, F., 2010. Molecular Mechanisms of SERT in Platelets: Regulation of Plasma Serotonin Levels. Mol Interv 10, 231–241. https://doi.org/10.1124/mi.10.4.6

Merten, M., Chow, T., Hellums, J.D., Thiagarajan, P., 2000. A new role for P-selectin in shear-induced platelet aggregation. Circulation 102, 2045–2050. https://doi.org/10.1161/01.cir.102.17.2045

Merten, M., Pakala, R., Thiagarajan, P., Benedict, C.R., 1999. Platelet microparticles promote platelet interaction with subendothelial matrix in a glycoprotein IIb/IIIa-dependent mechanism. Circulation 99, 2577–2582. https://doi.org/10.1161/01.cir.99.19.2577

Miller, V.M., Black, D.M., Brinton, E.A., Budoff, M.J., Cedars, M.I., Hodis, H.N., Lobo, R.A., Manson, J.E., Merriam, G.R., Naftolin, F., Santoro, N., Taylor, H.S., Harman, S.M., 2009. Using basic science to design a clinical trial: baseline characteristics of women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). J Cardiovasc Transl Res 2, 228–239. https://doi.org/10.1007/s12265-009-9104-y

Miller, V.M., Duckles, S.P., 2008. Vascular Actions of Estrogens: Functional Implications. Pharmacological Reviews 60, 210–241. https://doi.org/10.1124/pr.107.08002

Miner, J., Hoffhines, A., 2007. The Discovery of Aspirin's Antithrombotic Effects. Tex Heart Inst J 34, 179–186.

Mitchell, J.A., Ali, F., Bailey, L., Moreno, L., Harrington, L.S., 2008. Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. Exp Physiol 93, 141–147. https://doi.org/10.1113/expphysiol.2007.038588

Mitchell, M.D., 1978. A sensitive radioimmunoassay for 6-keto-prostaglandin F1α: preliminary observations on circulating concentrations. Prostaglandins and Medicine 1, 13–21. https://doi.org/10.1016/0161-4630(78)90072-1

Montero, D., Lundby, C., 2017. Refuting the myth of non-response to exercise training: 'non-responders' do respond to higher dose of training. J Physiol 595, 3377–3387. https://doi.org/10.1113/JP273480

Moreau, K.L., Hildreth, K.L., 2014. Vascular Aging across the Menopause Transition in Healthy Women. Advances in Vascular Medicine 2014, 1–12. https://doi.org/10.1155/2014/204390

Moreau, K.L., Stauffer, B.L., Kohrt, W.M., Seals, D.R., 2013. Essential Role of Estrogen for Improvements in Vascular Endothelial Function With Endurance Exercise in Postmenopausal Women. J Clin Endocrinol Metab 98, 4507–4515. https://doi.org/10.1210/jc.2013-2183

Muscella, A., Stefàno, E., Marsigliante, S., 2020. The effects of exercise training on lipid metabolism and coronary heart disease. Am J Physiol Heart Circ Physiol 319, H76–H88. https://doi.org/10.1152/ajpheart.00708.2019

Naci, H., Ioannidis, J.P.A., 2013. Comparative effectiveness of exercise and drug interventions on mortality outcomes: metaepidemiological study. BMJ : British Medical Journal (Online) 347. https://doi.org/10.1136/bmj.f5577

Nadal, A., Díaz, M., Valverde, M.A., 2001. The Estrogen Trinity: Membrane, Cytosolic, and Nuclear Effects. Physiology 16, 251–255. https://doi.org/10.1152/physiologyonline.2001.16.6.251

Nauman, J., Tauschek, L.C., Kaminsky, L.A., Nes, B.M., Wisløff, U., 2017. Global Fitness Levels: Findings From a Web-Based Surveillance Report. Progress in Cardiovascular Diseases, Physical Activity, Exercise and Fitness in Health and Disease 60, 78–88. https://doi.org/10.1016/j.pcad.2017.01.009

Nelson, H.D., Humphrey, L.L., Nygren, P., Teutsch, S.M., Allan, J.D., 2002. Postmenopausal hormone replacement therapy: scientific review. JAMA 288, 872–881. https://doi.org/10.1001/jama.288.7.872

Nestel, P.J., Pomeroy, S., Kay, S., Komesaroff, P., Behrsing, J., Cameron, J.D., West, L., 1999. Isoflavones from red clover improve systemic arterial compliance but not plasma lipids in menopausal women. J Clin Endocrinol Metab 84, 895–898. https://doi.org/10.1210/jcem.84.3.5561

Nishiyama, S.K., Wray, D.W., Richardson, R.S., 2008. Aging affects vascular structure and function in a limb-specific manner. J Appl Physiol (1985) 105, 1661–1670. https://doi.org/10.1152/japplphysiol.90612.2008

Norgard, N.B., Abu-Fadel, M., 2009. Comparison of prasugrel and clopidogrel in patients with acute coronary syndrome undergoing percutaneous coronary intervention. Vasc Health Risk Manag 5, 873–882.

Novella, S., Heras, M., Hermenegildo, C., Dantas, A.P., 2012. Effects of Estrogen on Vascular Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology 32, 2035–2042. https://doi.org/10.1161/ATVBAHA.112.250308

Novensà, L., Novella, S., Medina, P., Segarra, G., Castillo, N., Heras, M., Hermenegildo, C., Dantas, A.P., 2011. Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ERα/ERβ balance in female mice. PLoS One 6, e25335. https://doi.org/10.1371/journal.pone.0025335

Nyberg, M., Blackwell, J.R., Damsgaard, R., Jones, A.M., Hellsten, Y., Mortensen, S.P., 2012. Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans. The Journal of Physiology 590, 5361–5370. https://doi.org/10.1113/jphysiol.2012.239053

Nyberg, M., Egelund, J., Mandrup, C.M., Andersen, C.B., Hansen, K.M.B.E., Hergel, I.-M.F., Valbak-Andersen, N., Frikke-Schmidt, R., Stallknecht, B., Bangsbo, J., Hellsten, Y., 2017. Leg vascular and skeletal muscle mitochondrial adaptations to aerobic high-intensity exercise training are enhanced in the early postmenopausal phase. J Physiol 595, 2969–2983. https://doi.org/10.1113/JP273871

Nyberg, M., Egelund, J., Mandrup, C.M., Nielsen, M.B., Mogensen, A.S., Stallknecht, B., Bangsbo, J., Hellsten, Y., 2016. Early Postmenopausal Phase Is Associated With Reduced Prostacyclin-Induced Vasodilation That Is Reversed by Exercise Training. Hypertension 68, 1011–1020. https://doi.org/10.1161/HYPERTENSIONAHA.116.07866

Nyberg, M., Seidelin, K., Andersen, T.R., Overby, N.N., Hellsten, Y., Bangsbo, J., 2014. Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise training. Am J Physiol Regul Integr Comp Physiol 306, R510-517. https://doi.org/10.1152/ajpregu.00539.2013

Nystoriak, M.A., Bhatnagar, A., 2018. Cardiovascular Effects and Benefits of Exercise. Front Cardiovasc Med 5, 135. https://doi.org/10.3389/fcvm.2018.00135

Ojeda, S.R., Prevot, V., Heger, S., Lomniczi, A., Dziedzic, B., Mungenast, A., 2003. The neurobiology of female puberty. Horm Res 60 Suppl 3, 15–20. https://doi.org/10.1159/000074495

Olvera Lopez, E., Ballard, B.D., Jan, A., 2022. Cardiovascular Disease, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Ozemek, C., Laddu, D.R., Lavie, C.J., Claeys, H., Kaminsky, L.A., Ross, R., Wisloff, U., Arena, R., Blair, S.N., 2018. An Update on the Role of Cardiorespiratory Fitness, Structured Exercise and Lifestyle Physical Activity in Preventing Cardiovascular Disease and Health Risk. Progress in Cardiovascular Diseases, Advances in Echocardiography 61, 484–490. https://doi.org/10.1016/j.pcad.2018.11.005

Palta, S., Saroa, R., Palta, A., 2014. Overview of the coagulation system. Indian J Anaesth 58, 515–523. https://doi.org/10.4103/0019-5049.144643

Park, Y.-M., Pereira, R.I., Erickson, C.B., Swibas, T.A., Kang, C., Van Pelt, R.E., 2017. Time since menopause and skeletal muscle estrogen receptors, PGC-1α, and AMPK. Menopause 24, 815–823. https://doi.org/10.1097/GME.0000000000000829

Parker, B.A., Kalasky, M.J., Proctor, D.N., 2010. Evidence for sex differences in cardiovascular aging and adaptive responses to physical activity. Eur J Appl Physiol 110, 235–246. https://doi.org/10.1007/s00421-010-1506-7

Parker, W.A.E., Orme, R.C., Hanson, J., Stokes, H.M., Bridge, C.M., Shaw, P.A., Sumaya, W., Thorneycroft, K., Petrucci, G., Porro, B., Judge, H.M., Ajjan, R.A., Rocca, B., Storey, R.F., 2019. Very-low-dose twice-daily aspirin maintains platelet inhibition and improves haemostasis during dual-antiplatelet therapy for acute coronary syndrome. Platelets 30, 148–157. https://doi.org/10.1080/09537104.2019.1572880

Pedersen, M.T., Nørregaard, L.B., Jensen, T.D., Frederiksen, A.S., Ottesen, L., Bangsbo, J., 2022. The effect of 5 years of team sport on elderly males' health and social capital-An interdisciplinary follow-up study. Health Sci Rep 5, e760. https://doi.org/10.1002/hsr2.760

Pedersen, M.T., Vorup, J., Bangsbo, J., 2018. Effect of a 26-month floorball training on male elderly's cardiovascular fitness, glucose control, body composition, and functional capacity. J Sport Health Sci 7, 149–158. https://doi.org/10.1016/j.jshs.2017.12.002

Periayah, M.H., Halim, A.S., Mat Saad, A.Z., 2017. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. Int J Hematol Oncol Stem Cell Res 11, 319–327.

Perry, M.-C., Dufour, C.R., Tam, I.S., B'chir, W., Giguère, V., 2014. Estrogen-related receptor-α coordinates transcriptional programs essential for exercise tolerance and muscle fitness. Mol Endocrinol 28, 2060–2071. https://doi.org/10.1210/me.2014-1281

Pfitscher, A., Reiter, E., Jungbauer, A., 2008. Receptor binding and transactivation activities of red clover isoflavones and their metabolites. J Steroid Biochem Mol Biol 112, 87–94. https://doi.org/10.1016/j.jsbmb.2008.08.007

Phillips, D.R., 1985. Receptors for Platelet Agonists, in: George, J.N., Nurden, A.T., Phillips, D.R. (Eds.), Platelet Membrane Glycoproteins. Springer US, Boston, MA, pp. 145–169. https://doi.org/10.1007/978-1-4684-4880-1_7

Pierce, G.L., Eskurza, I., Walker, A.E., Fay, T.N., Seals, D.R., 2011. Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults. Clin Sci (Lond) 120, 13–23. https://doi.org/10.1042/CS20100174

Pignatelli, P., De Biase, L., Lenti, L., Tocci, G., Brunelli, A., Cangemi, R., Riondino, S., Grego, S., Volpe, M., Violi, F., 2005. Tumor necrosis factor-α as trigger of platelet activation in patients with heart failure. Blood 106, 1992–1994. https://doi.org/10.1182/blood-2005-03-1247

Pilote, L., Raparelli, V., 2018. Participation of Women in Clinical Trials: Not Yet Time to Rest on Our Laurels∗. Journal of the American College of Cardiology 71, 1970–1972. https://doi.org/10.1016/j.jacc.2018.02.069

Pilšáková, L., Riečanský, I., Jagla, F., 2010. The physiological actions of isoflavone phytoestrogens. Physiol Res 59, 651–664. https://doi.org/10.33549/physiolres.931902

Polo-Kantola, P., Erkkola, R., Irjala, K., Pullinen, S., Virtanen, I., Polo, O., 1999. Effect of short-term transdermal estrogen replacement therapy on sleep: a randomized, double-blind crossover trial in postmenopausal women. Fertility and Sterility 71, 873–880. https://doi.org/10.1016/S0015-0282(99)00062-X

Pomeroy, J.E., Helfer, A., Bursac, N., 2020. Biomaterializing the Promise of Cardiac Tissue Engineering. Biotechnol Adv 42, 107353. https://doi.org/10.1016/j.biotechadv.2019.02.009

Prior, J.C., 2020. Women's reproductive system as balanced estradiol and progesterone actions—A revolutionary, paradigm-shifting concept in women's health. Drug Discovery Today: Disease Models, Special section on Skin disease and Women's health 32, 31–40. https://doi.org/10.1016/j.ddmod.2020.11.005

Prossnitz, E.R., Barton, M., 2011. The G-protein-coupled estrogen receptor GPER in health and disease. Nat Rev Endocrinol 7, 715–726. https://doi.org/10.1038/nrendo.2011.122

Puca, A.A., Carrizzo, A., Villa, F., Ferrario, A., Casaburo, M., Maciąg, A., Vecchione, C., 2013. Vascular ageing: the role of oxidative stress. Int J Biochem Cell Biol 45, 556–559. https://doi.org/10.1016/j.biocel.2012.12.024

Rademaker, M., 2001. Do women have more adverse drug reactions? Am J Clin Dermatol 2, 349–351. https://doi.org/10.2165/00128071-200102060-00001

Rahman, M.M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., Almikhlafi, M.A., Alghamdi, S.Q., Alruwaili, A.S., Hossain, M.S., Ahmed, M., Das, R., Emran, T.B., Uddin, M.S., 2022. Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules 27, 233. https://doi.org/10.3390/molecules27010233

Randolph, J.F., Zheng, H., Sowers, M.R., Crandall, C., Crawford, S., Gold, E.B., Vuga, M., 2011. Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. J Clin Endocrinol Metab 96, 746–754. https://doi.org/10.1210/jc.2010-1746

Reed, B.G., Carr, B.R., 2018. The Normal Menstrual Cycle and the Control of Ovulation. MDText.com, Inc., Massachusetts.

Reuter, I., 2012. Aging, Physical Activity, and Disease Prevention 2012. J Aging Res 2012, 373294. https://doi.org/10.1155/2012/373294

Ribeiro, I. c., de Lucena, E. g. p., Oliveira, A. s., Fernandes, P. t., 2022. Physical exercise in menopause: a review of physiological changes. Comparative Exercise Physiology 18, 275–279. https://doi.org/10.3920/CEP220009

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Adams, R.J., Berry, J.D., Brown, T.M., Carnethon, M.R., Dai, S., de Simone, G., Ford, E.S., Fox, C.S., Fullerton, H.J., Gillespie, C., Greenlund, K.J., Hailpern, S.M., Heit, J.A., Ho, P.M., Howard, V.J., Kissela, B.M., Kittner, S.J., Lackland, D.T., Lichtman, J.H., Lisabeth, L.D., Makuc, D.M., Marcus, G.M., Marelli, A., Matchar, D.B., McDermott, M.M., Meigs, J.B., Moy, C.S., Mozaffarian, D., Mussolino, M.E., Nichol, G., Paynter, N.P., Rosamond, W.D., Sorlie, P.D., Stafford, R.S., Turan, T.N., Turner, M.B., Wong, N.D., Wylie-Rosett, J., 2011. Heart Disease and Stroke Statistics—2011 Update. Circulation 123, e18–e209. https://doi.org/10.1161/CIR.0b013e3182009701

Rosano, G.M.C., Vitale, C., Fini, M., 2009. Cardiovascular aspects of menopausal hormone replacement therapy. Climacteric 12, 41–46. https://doi.org/10.1080/13697130903012306

Rosenberg, R.D., Rosenberg, J.S., 1984. Natural anticoagulant mechanisms. J Clin Invest 74, 1–6.

Ross, R., 1999. Atherosclerosis--an inflammatory disease. N Engl J Med 340, 115–126. https://doi.org/10.1056/NEJM199901143400207

Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A.A., Howard, B.V., Johnson, K.C., Kotchen, J.M., Ockene, J., Writing Group for the Women's Health Initiative Investigators, 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 288, 321–333. https://doi.org/10.1001/jama.288.3.321

Rossouw, J.E., Prentice, R.L., Manson, J.E., Wu, L., Barad, D., Barnabei, V.M., Ko, M., LaCroix, A.Z., Margolis, K.L., Stefanick, M.L., 2007. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA 297, 1465–1477. https://doi.org/10.1001/jama.297.13.1465

Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noël C., Beaton, A.Z., Benjamin, E.J., Benziger, C.P., Bonny, Aimé, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J., Catapano, A.L., Chugh, S.S., Cooper, L.T., Coresh, J., Criqui, M., DeCleene, N., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Solà, J., Fowkes, G., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N., Koroshetz, W., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Temesgen, A.M., Mokdad, A., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Moraes de Oliveira, G., Otto, C., Owolabi, M., Pratt, M., Rajagopalan, S., Reitsma, M., Ribeiro, A.L.P., Rigotti, N., Rodgers, A., Sable, C., Shakil, S., Sliwa-Hahnle, K., Stark, B., Sundström, J., Timpel, P., Tleyjeh, I.M., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L., Murray, C., Fuster, V., Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, Noel C., Beaton, A., Benjamin, E.J., Benziger, C.P., Bonny, Aime, Brauer, M., Brodmann, M., Cahill, T.J., Carapetis, J.R., Catapano, A.L., Chugh, S., Cooper, L.T., Coresh, J., Criqui, M.H., DeCleene, N.K., Eagle, K.A., Emmons-Bell, S., Feigin, V.L., Fernández-Sola, J., Fowkes, F.G.R., Gakidou, E., Grundy, S.M., He, F.J., Howard, G., Hu, F., Inker, L., Karthikeyan, G., Kassebaum, N.J., Koroshetz, W.J., Lavie, C., Lloyd-Jones, D., Lu, H.S., Mirijello, A., Misganaw, A.T., Mokdad, A.H., Moran, A.E., Muntner, P., Narula, J., Neal, B., Ntsekhe, M., Oliveira, G.M.M., Otto, C.M., Owolabi, M.O., Pratt, M., Rajagopalan, S., Reitsma, M.B., Ribeiro, A.L.P., Rigotti, N.A., Rodgers, A., Sable, C.A., Shakil, S.S., Sliwa, K., Stark, B.A., Sundström, J., Timpel, P., Tleyjeh, I.I., Valgimigli, M., Vos, T., Whelton, P.K., Yacoub, M., Zuhlke, L.J., Abbasi-Kangevari, M., Abdi, A., Abedi, A., Aboyans, V., Abrha, W.A., Abu-Gharbieh, E., Abushouk, A.I., Acharya, D., Adair, T., Adebayo, O.M., Ademi, Z., Advani, S.M., Afshari, K., Afshin, A., Agarwal, G., Agasthi, P., Ahmad, S., Ahmadi, S., Ahmed, M.B., Aji, B., Akalu, Y., Akande-Sholabi, W., Aklilu, A., Akunna, C.J., Alahdab, F., Al-Eyadhy, A., Alhabib, K.F., Alif, S.M., Alipour, V., Aljunid, S.M., Alla, F., Almasi-Hashiani, A., Almustanyir, S., Al-Raddadi, R.M., Amegah, A.K., Amini, S., Aminorroaya, A., Amu, H., Amugsi, D.A., Ancuceanu, R., Anderlini, D., Andrei, T., Andrei, C.L., Ansari-Moghaddam, A., Anteneh, Z.A., Antonazzo, I.C., Antony, B., Anwer, R., Appiah, L.T., Arabloo, J., Ärnlöv, J., Artanti, K.D., Ataro, Z., Ausloos, M., Avila-Burgos, L., Awan, A.T., Awoke, M.A., Ayele, H.T., Ayza, M.A., Azari, S., B, D.B., Baheiraei, N., Baig, A.A., Bakhtiari, A., Banach, M., Banik, P.C., Baptista, E.A., Barboza, M.A., Barua, L., Basu, S., Bedi, N., Béjot, Y., Bennett, D.A., Bensenor, I.M., Berman, A.E., Bezabih, Y.M., Bhagavathula, A.S., Bhaskar, S., Bhattacharyya, K., Bijani, A., Bikbov, B., Birhanu, M.M., Boloor, A., Brant, L.C., Brenner, H., Briko, N.I., Butt, Z.A., Caetano dos Santos, F.L., Cahill, L.E., Cahuana-Hurtado, L., Cámera, L.A., Campos-Nonato, I.R., Cantu-Brito, C., Car, J., Carrero, J.J., Carvalho, F., Castañeda-Orjuela, C.A., Catalá-López, F., Cerin, E., Charan, J., Chattu, V.K., Chen, S., Chin, K.L., Choi, J.-Y.J., Chu, D.-T., Chung, S.-C., Cirillo, M., Coffey, S., Conti, S., Costa, V.M., Cundiff, D.K., Dadras, O., Dagnew, B., Dai, X., Damasceno, A.A.M., Dandona, L., Dandona, R., Davletov, K., De la Cruz-Góngora, V., De la Hoz, F.P., De Neve, J.-W., Denova-Gutiérrez, E., Derbew Molla, M., Derseh, B.T.,

Desai, R., Deuschl, G., Dharmaratne, S.D., Dhimal, M., Dhungana, R.R., Dianatinasab, M., Diaz, D., Djalalinia, S., Dokova, K., Douiri, A., Duncan, B.B., Duraes, A.R., Eagan, A.W., Ebtehaj, S., Eftekhari, A., Eftekharzadeh, S., Ekholuenetale, M., El Nahas, N., Elgendy, I.Y., Elhadi, M., El-Jaafary, S.I., Esteghamati, S., Etisso, A.E., Eyawo, O., Fadhil, I., Faraon, E.J.A., Faris, P.S., Farwati, M., Farzadfar, F., Fernandes, E., Fernandez Prendes, C., Ferrara, P., Filip, I., Fischer, F., Flood, D., Fukumoto, T., Gad, M.M., Gaidhane, S., Ganji, M., Garg, J., Gebre, A.K., Gebregiorgis, B.G., Gebregzabiher, K.Z., Gebremeskel, G.G., Getacher, L., Obsa, A.G., Ghajar, A., Ghashghaee, A., Ghith, N., Giampaoli, S., Gilani, S.A., Gill, P.S., Gillum, R.F., Glushkova, E.V., Gnedovskaya, E.V., Golechha, M., Gonfa, K.B., Goudarzian, A.H., Goulart, A.C., Guadamuz, J.S., Guha, A., Guo, Y., Gupta, R., Hachinski, V., Hafezi-Nejad, N., Haile, T.G., Hamadeh, R.R., Hamidi, S., Hankey, G.J., Hargono, A., Hartono, R.K., Hashemian, M., Hashi, A., Hassan, S., Hassen, H.Y., Havmoeller, R.J., Hay, S.I., Hayat, K., Heidari, G., Herteliu, C., Holla, R., Hosseini, M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Househ, M., Huang, J., Humayun, A., Iavicoli, I., Ibeneme, C.U., Ibitoye, S.E., Ilesanmi, O.S., Ilic, I.M., Ilic, M.D., Iqbal, U., Irvani, S.S.N., Islam, S.M.S., Islam, R.M., Iso, H., Iwagami, M., Jain, V., Javaheri, T., Jayapal, S.K., Jayaram, S., Jayawardena, R., Jeemon, P., Jha, R.P., Jonas, J.B., Jonnagaddala, J., Joukar, F., Jozwiak, J.J., Jürisson, M., Kabir, A., Kahlon, T., Kalani, R., Kalhor, R., Kamath, A., Kamel, I., Kandel, H., Kandel, A., Karch, A., Kasa, A.S., Katoto, P.D.M.C., Kayode, G.A., Khader, Y.S., Khammarnia, M., Khan, M.S., Khan, M.N., Khan, M., Khan, E.A., Khatab, K., Kibria, G.M.A., Kim, Y.J., Kim, G.R., Kimokoti, R.W., Kisa, S., Kisa, A., Kivimäki, M., Kolte, D., Koolivand, A., Korshunov, V.A., Koulmane Laxminarayana, S.L., Koyanagi, A., Krishan, K., Krishnamoorthy, V., Kuate Defo, B., Kucuk Bicer, B., Kulkarni, V., Kumar, G.A., Kumar, N., Kurmi, O.P., Kusuma, D., Kwan, G.F., La Vecchia, C., Lacey, B., Lallukka, T., Lan, Q., Lasrado, S., Lassi, Z.S., Lauriola, P., Lawrence, W.R., Laxmaiah, A., LeGrand, K.E., Li, M.-C., Li, B., Li, S., Lim, S.S., Lim, L.-L., Lin, H., Lin, Z., Lin, R.-T., Liu, X., Lopez, A.D., Lorkowski, S., Lotufo, P.A., Lugo, A., M, N.K., Madotto, F., Mahmoudi, M., Majeed, A., Malekzadeh, R., Malik, A.A., Mamun, A.A., Manafi, N., Mansournia, M.A., Mantovani, L.G., Martini, S., Mathur, M.R., Mazzaglia, G., Mehata, S., Mehndiratta, M.M., Meier, T., Menezes, R.G., Meretoja, A., Mestrovic, T., Miazgowski, B., Miazgowski, T., Michalek, I.M., Miller, T.R., Mirrakhimov, E.M., Mirzaei, H., Moazen, B., Moghadaszadeh, M., Mohammad, Y., Mohammad, D.K., Mohammed, S., Mohammed, M.A., Mokhayeri, Y., Molokhia, M., Montasir, A.A., Moradi, G., Moradzadeh, R., Moraga, P., Morawska, L., Moreno Velásquez, I., Morze, J., Mubarik, S., Muruet, W., Musa, K.I., Nagarajan, A.J., Nalini, M., Nangia, V., Naqvi, A.A., Narasimha Swamy, S., Nascimento, B.R., Nayak, V.C., Nazari, J., Nazarzadeh, M., Negoi, R.I., Neupane Kandel, S., Nguyen, H.L.T., Nixon, M.R., Norrving, B., Noubiap, J.J., Nouthe, B.E., Nowak, C., Odukoya, O.O., Ogbo, F.A., Olagunju, A.T., Orru, H., Ortiz, A., Ostroff, S.M., Padubidri, J.R., Palladino, R., Pana, A., Panda-Jonas, S., Parekh, U., Park, E.-C., Parvizi, M., Pashazadeh Kan, F., Patel, U.K., Pathak, M., Paudel, R., Pepito, V.C.F., Perianayagam, A., Perico, N., Pham, H.Q., Pilgrim, T., Piradov, M.A.,

Pishgar, F., Podder, V., Polibin, R.V., Pourshams, A., Pribadi, D.R.A., Rabiee, N., Rabiee, M., Radfar, A., Rafiei, A., Rahim, F., Rahimi-Movaghar, V., Ur Rahman, M.H., Rahman, M.A., Rahmani, A.M., Rakovac, I., Ram, P., Ramalingam, S., Rana, J., Ranasinghe, P., Rao, S.J., Rathi, P., Rawal, L., Rawasia, W.F., Rawassizadeh, R., Remuzzi, G., Renzaho, A.M.N., Rezapour, A., Riahi, S.M., Roberts-Thomson, R.L., Roever, L., Rohloff, P., Romoli, M., Roshandel, G., Rwegerera, G.M., Saadatagah, S., Saber-Ayad, M.M., Sabour, S., Sacco, S., Sadeghi, M., Saeedi Moghaddam, S., Safari, S., Sahebkar, A., Salehi, S., Salimzadeh, H., Samaei, M., Samy, A.M., Santos, I.S., Santric-Milicevic, M.M., Sarrafzadegan, N., Sarveazad, A., Sathish, T., Sawhney, M., Saylan, M., Schmidt, M.I., Schutte, A.E., Senthilkumaran, S., Sepanlou, S.G., Sha, F., Shahabi, S., Shahid, I., Shaikh, M.A., Shamali, M., Shamsizadeh, M., Shawon, M.S.R., Sheikh, A., Shigematsu, M., Shin, M.-J., Shin, J.I., Shiri, R., Shiue, I., Shuval, K., Siabani, S., Siddiqi, T.J., Silva, D.A.S., Singh, J.A., Mtech, A.S., Skryabin, V.Y., Skryabina, A.A., Soheili, A., Spurlock, E.E., Stockfelt, L., Stortecky, S., Stranges, S., Suliankatchi Abdulkader, R., Tadbiri, H., Tadesse, E.G., Tadesse, D.B., Tajdini, M., Tariqujjaman, M., Teklehaimanot, B.F., Temsah, M.-H., Tesema, A.K., Thakur, B., Thankappan, K.R., Thapar, R., Thrift, A.G., Timalsina, B., Tonelli, M., Touvier, M., Tovani-Palone, M.R., Tripathi, A., Tripathy, J.P., Truelsen, T.C., Tsegay, G.M., Tsegaye, G.W., Tsilimparis, N., Tusa, B.S., Tyrovolas, S., Umapathi, K.K., Unim, B., Unnikrishnan, B., Usman, M.S., Vaduganathan, M., Valdez, P.R., Vasankari, T.J., Velazquez, D.Z., Venketasubramanian, N., Vu, G.T., Vujcic, I.S., Waheed, Y., Wang, Y., Wang, F., Wei, J., Weintraub, R.G., Weldemariam, A.H., Westerman, R., Winkler, A.S., Wiysonge, C.S., Wolfe, C.D.A., Wubishet, B.L., Xu, G., Yadollahpour, A., Yamagishi, K., Yan, L.L., Yandrapalli, S., Yano, Y., Yatsuya, H., Yeheyis, T.Y., Yeshaw, Y., Yilgwan, C.S., Yonemoto, N., Yu, C., Yusefzadeh, H., Zachariah, G., Zaman, S.B., Zaman, M.S., Zamanian, M., Zand, R., Zandifar, A., Zarghi, A., Zastrozhin, M.S., Zastrozhina, A., Zhang, Z.-J., Zhang, Y., Zhang, W., Zhong, C., Zou, Z., Zuniga, Y.M.H., Murray, C.J.L., Fuster, V., 2020. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology 76, 2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010

Sabra, A., Lawrence, M.J., Aubrey, R., Obaid, D.R., Chase, A.J., Smith, D., Thomas, P., Storton, S., Davies, G., Hawkins, K., Morris, K., Evans, P.A., 2017. Characterisation of clot microstructure properties in stable coronary artery disease | Open Heart [WWW Document]. URL https://openheart.bmj.com/content/4/2/e000562.abstract (accessed 9.18.22).

Sadler, J.E., 1998. Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem 67, 395–424. https://doi.org/10.1146/annurev.biochem.67.1.395

Samuels, C., 2009. Sleep, recovery, and performance: the new frontier in high-performance athletics. Phys Med Rehabil Clin N Am 20, 149–159, ix. https://doi.org/10.1016/j.pmr.2008.10.009

Santoro, N., Epperson, C.N., Mathews, S.B., 2015. Menopausal Symptoms and Their Management. Endocrinol Metab Clin North Am 44, 497–515. https://doi.org/10.1016/j.ecl.2015.05.001

Santoro, N., Randolph, J.F., 2011. Reproductive Hormones and the Menopause Transition. Obstet Gynecol Clin North Am 38, 455–466. https://doi.org/10.1016/j.ogc.2011.05.004

Santos-Parker, J.R., Strahler, T.R., Vorwald, V.M., Pierce, G.L., Seals, D.R., 2017. Habitual aerobic exercise does not protect against micro- or macrovascular endothelial dysfunction in healthy estrogen-deficient postmenopausal women. J Appl Physiol (1985) 122, 11–19. https://doi.org/10.1152/japplphysiol.00732.2016

Sathyapalan, T., Aye, M., Rigby, A.S., Thatcher, N.J., Dargham, S.R., Kilpatrick, E.S., Atkin, S.L., 2018. Soy isoflavones improve cardiovascular disease risk markers in women during the early menopause. Nutr Metab Cardiovasc Dis 28, 691–697. https://doi.org/10.1016/j.numecd.2018.03.007

Savi, P., Nurden, P., Nurden, A.T., Levy-Toledano, S., Herbert, J.M., 1998. Clopidogrel: a review of its mechanism of action. Platelets 9, 251–255. https://doi.org/10.1080/09537109876799

Scharhag-Rosenberger, F., Meyer, T., Gässler, N., Faude, O., Kindermann, W., 2010. Exercise at given percentages of VO2max: heterogeneous metabolic responses between individuals. J Sci Med Sport 13, 74–79. https://doi.org/10.1016/j.jsams.2008.12.626

Schmaier, A.H., 2008. The elusive physiologic role of Factor XII. J Clin Invest 118, 3006–3009. https://doi.org/10.1172/JCI36617

Schmidt, J.F., Hansen, P.R., Andersen, T.R., Andersen, L.J., Hornstrup, T., Krustrup, P., Bangsbo, J., 2014. Cardiovascular adaptations to 4 and 12 months of football or strength training in 65- to 75-year-old untrained men. Scand J Med Sci Sports 24 Suppl 1, 86–97. https://doi.org/10.1111/sms.12217

Schreiber, S.N., Emter, R., Hock, M.B., Knutti, D., Cardenas, J., Podvinec, M., Oakeley, E.J., Kralli, A., 2004. The estrogen-related receptor α (ERRα) functions in PPARγ coactivator 1α (PGC-1α)-induced mitochondrial biogenesis. Proceedings of the National Academy of Sciences 101, 6472–6477. https://doi.org/10.1073/pnas.0308686101

Schuff-Werner, P., Steiner, M., Fenger, S., Gross, H.-J., Bierlich, A., Dreissiger, K., Mannuß, S., Siegert, G., Bachem, M., Kohlschein, P., 2013. Effective estimation of correct platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. Br J Haematol 162, 684–692. https://doi.org/10.1111/bjh.12443

Seals, D.R., Jablonski, K.L., Donato, A.J., 2011. Aging and vascular endothelial function in humans. Clinical Science 120, 357–375. https://doi.org/10.1042/CS20100476

Seidelin, K., Nyberg, M., Piil, P., Jørgensen, N.R., Hellsten, Y., Bangsbo, J., 2017. Adaptations with Intermittent Exercise Training in Post- and Premenopausal Women. Medicine & Science in Sports & Exercise 49, 96–105. https://doi.org/10.1249/MSS.0000000000001071

Shibata, S., Fujimoto, N., Hastings, J.L., Carrick-Ranson, G., Bhella, P.S., Hearon Jr., C.M., Levine, B.D., 2018. The effect of lifelong exercise frequency on arterial stiffness. The Journal of Physiology 596, 2783–2795. https://doi.org/10.1113/JP275301

Simoncini, T., Garibaldi, S., Fu, X.-D., Pisaneschi, S., Begliuomini, S., Baldacci, C., Lenzi, E., Goglia, L., Giretti, M.S., Genazzani, A.R., 2008. Effects of phytoestrogens derived from red clover on atherogenic adhesion molecules in human endothelial cells. Menopause 15, 542–550. https://doi.org/10.1097/gme.0b013e318156f9d6

Smith, E.S., McKay, A.K.A., Kuikman, M., Ackerman, K.E., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., Burke, L.M., 2022. Auditing the Representation of Female Versus Male Athletes in Sports Science and Sports Medicine Research: Evidence-Based Performance Supplements. Nutrients 14, 953. https://doi.org/10.3390/nu14050953

Smith, L.L., 2000. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? Med Sci Sports Exerc 32, 317–331. https://doi.org/10.1097/00005768-200002000-00011

Sowers, M.R., Eyvazzadeh, A.D., McConnell, D., Yosef, M., Jannausch, M.L., Zhang, D., Harlow, S., Randolph, J.F., 2008. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab 93, 3478–3483. https://doi.org/10.1210/jc.2008-0567

Spalding, A., Vaitkevicius, H., Dill, S., MacKenzie, S., Schmaier, A., Lockette, W., 1998. Mechanism of epinephrine-induced platelet aggregation. Hypertension 31, 603–607. https://doi.org/10.1161/01.hyp.31.2.603

Spina, R.J., Ogawa, T., Kohrt, W.M., Martin, W.H., Holloszy, J.O., Ehsani, A.A., 1993. Differences in cardiovascular adaptations to endurance exercise training between older men and women. J Appl Physiol (1985) 75, 849–855. https://doi.org/10.1152/jappl.1993.75.2.849

Squadrito, F., Altavilla, D., Morabito, N., Crisafulli, A., D'Anna, R., Corrado, F., Ruggeri, P., Campo, G.M., Calapai, G., Caputi, A.P., Squadrito, G., 2002. The effect of the phytoestrogen genistein on plasma nitric oxide concentrations, endothelin-1 levels and endothelium dependent vasodilation in postmenopausal women. Atherosclerosis 163, 339–347. https://doi.org/10.1016/S0021-9150(02)00013-8

Staessen, J.A., Ginocchio, G., Thijs, L., Fagard, R., 1997. Conventional and ambulatory blood pressure and menopause in a prospective population study. J Hum Hypertens 11, 507–514. https://doi.org/10.1038/sj.jhh.1000476

Stokes, K.Y., Granger, D.N., 2012. Platelets: a critical link between inflammation and microvascular dysfunction. J Physiol 590, 1023–1034. https://doi.org/10.1113/jphysiol.2011.225417

Su, H.I., Freeman, E.W., 2009. Hormone changes associated with the menopausal transition. Minerva Ginecol 61, 483–489.

Thomas, P., Pang, Y., 2013. Protective actions of progesterone in the cardiovascular system: potential role of membrane progesterone receptors (mPRs) in mediating rapid effects. Steroids 78, 583–588. https://doi.org/10.1016/j.steroids.2013.01.003

Thompson, W.R., Sallis, R., Joy, E., Jaworski, C.A., Stuhr, R.M., Trilk, J.L., 2020. Exercise Is Medicine. American Journal of Lifestyle Medicine 14, 511–523. https://doi.org/10.1177/1559827620912192

Thorp, J.A., Walsh, S.W., Brath, P.C., 1988. Low-dose aspirin inhibits thromboxane, but not prostacyclin, production by human placental arteries. Am J Obstet Gynecol 159, 1381–1384. https://doi.org/10.1016/0002-9378(88)90560-1

Thorup, A.C., Lambert, M.N., Kahr, H.S., Bjerre, M., Jeppesen, P.B., 2015. Intake of Novel Red Clover Supplementation for 12 Weeks Improves Bone Status in Healthy Menopausal Women. Evid Based Complement Alternat Med 2015, 689138. https://doi.org/10.1155/2015/689138

Timmons, J.A., Knudsen, S., Rankinen, T., Koch, L.G., Sarzynski, M., Jensen, T., Keller, P., Scheele, C., Vollaard, N.B.J., Nielsen, S., Akerström, T., MacDougald, O.A., Jansson, E., Greenhaff, P.L., Tarnopolsky, M.A., van Loon, L.J.C., Pedersen, B.K., Sundberg, C.J., Wahlestedt, C., Britton, S.L., Bouchard, C., 2010. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. J Appl Physiol (1985) 108, 1487–1496. https://doi.org/10.1152/japplphysiol.01295.2009

Ting, Y., Jiang, Y., Ho, C.-T., Huang, Q., 2014. Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals. Journal of Functional Foods 7, 112–128. https://doi.org/10.1016/j.jff.2013.12.010

Tofas, T., Draganidis, D., Deli, C.K., Georgakouli, K., Fatouros, I.G., Jamurtas, A.Z., 2020. Exercise-Induced Regulation of Redox Status in Cardiovascular Diseases: The Role of Exercise Training and Detraining. Antioxidants 9, 13. https://doi.org/10.3390/antiox9010013

Trifan, G., Gorelick, P.B., Testai, F.D., 2021. Efficacy and Safety of Using Dual Versus Monotherapy Antiplatelet Agents in Secondary Stroke Prevention: Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. Circulation 143, 2441–2453. https://doi.org/10.1161/CIRCULATIONAHA.121.053782

Tripathi, M., Yen, P.M., Singh, B.K., 2020. Estrogen-Related Receptor Alpha: An Under-Appreciated Potential Target for the Treatment of Metabolic Diseases. Int J Mol Sci 21, 1645. https://doi.org/10.3390/ijms21051645

Troy, G.C., 1988. An Overview of Hemostasis. Veterinary Clinics of North America: Small Animal Practice 18, 5–20. https://doi.org/10.1016/S0195-5616(88)50003-7

van Beek, J.H.G.M., Kirkwood, T.B.L., Bassingthwaighte, J.B., 2016. Understanding the physiology of the ageing individual: computational modelling of changes in metabolism and endurance. Interface Focus 6, 20150079. https://doi.org/10.1098/rsfs.2015.0079

Verdoia, M., Suryapranata, H., Damen, S., Camaro, C., Benit, E., Barbieri, L., Rasoul, S., Liew, H.B., Polad, J., Ahmad, W. a. W., Zambahari, R., Lalmand, J., van der Schaaf, R.J., Koh, T.H., Timmermans, P., Dilling-Boer, D., Veenstra, L.F., Van't Hof, A.W.J., Lee, S.W.L., Roolvink, V., Ligtenberg, E., Postma, S., Kolkman, E.J.J., Brouwer, M.A., Kedhi, E., De Luca, G., 2021. Gender differences with short-term vs 12 months dual antiplatelet therapy in patients with acute coronary syndrome treated with the COMBO dual therapy stent: 2-years follow-up results of the

REDUCE trial. J Thromb Thrombolysis 52, 797–807. https://doi.org/10.1007/s11239-021-02439-x
Viggiani, M.T., Polimeno, L., Di Leo, A., Barone, M., 2019. Phytoestrogens: Dietary Intake, Bioavailability, and Protective Mechanisms against Colorectal Neoproliferative Lesions. Nutrients 11, 1709. https://doi.org/10.3390/nu11081709
Villena, J.A., Kralli, A., 2008. ERRα: a metabolic function for the oldest orphan. Trends Endocrinol Metab 19, 269–276. https://doi.org/10.1016/j.tem.2008.07.005
Vitale, D.C., Piazza, C., Melilli, B., Drago, F., Salomone, S., 2013. Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet 38, 15–25. https://doi.org/10.1007/s13318-012-0112-y
Vorup, J., Pedersen, M.T., Brahe, L.K., Melcher, P.S., Alstrøm, J.M., Bangsbo, J., 2017. Effect of small-sided team sport training and protein intake on muscle mass, physical function and markers of health in older untrained adults: A randomized trial. PLoS One 12, e0186202. https://doi.org/10.1371/journal.pone.0186202
Wagner, P.D., 2011. The critical role of VEGF in skeletal muscle angiogenesis and blood flow. Biochem Soc Trans 39, 1556–1559. https://doi.org/10.1042/BST20110646
Wallace, R.B., Sherman, B.M., Bean, J.A., Treloar, A.E., Schlabaugh, L., 1979. Probability of menopause with increasing duration of amenorrhea in middle-aged women. Am J Obstet Gynecol 135, 1021–1024. https://doi.org/10.1016/0002-9378(79)90729-4
Wang, G.-R., Zhu, Y., Halushka, P.V., Lincoln, T.M., Mendelsohn, M.E., 1998. Mechanism of platelet inhibition by nitric oxide: In vivo phosphorylation of thromboxane receptor by cyclic GMP-dependent protein kinase. Proc Natl Acad Sci U S A 95, 4888–4893.
Warlo, E.M.K., Arnesen, H., Seljeflot, I., 2019. A brief review on resistance to P2Y12 receptor antagonism in coronary artery disease. Thrombosis Journal 17, 11. https://doi.org/10.1186/s12959-019-0197-5
Warner, T.D., Armstrong, P.C., Chan, M.V., Knowles, R.B., 2016. The importance of endothelium-derived mediators to the efficacy of dual anti-platelet therapy. Expert Rev Hematol 9, 223–225. https://doi.org/10.1586/17474086.2016.1140035
Warner, T.D., Nylander, S., Whatling, C., 2011. Anti-platelet therapy: cyclo-oxygenase inhibition and the use of aspirin with particular regard to dual anti-platelet therapy. Br J Clin Pharmacol 72, 619–633. https://doi.org/10.1111/j.1365-2125.2011.03943.x
Weisel, J.W., Litvinov, R.I., 2017. Fibrin Formation, Structure and Properties. Subcell Biochem 82, 405–456. https://doi.org/10.1007/978-3-319-49674-0_13
Welt, C.K., McNicholl, D.J., Taylor, A.E., Hall, J.E., 1999. Female reproductive aging is marked by decreased secretion of dimeric inhibin. J Clin Endocrinol Metab 84, 105–111. https://doi.org/10.1210/jcem.84.1.5381
Wendelboe, A.M., Raskob, G.E., 2016. Global Burden of Thrombosis. Circulation Research 118, 1340–1347. https://doi.org/10.1161/CIRCRESAHA.115.306841

Westby, C.M., Weil, B.R., Greiner, J.J., Stauffer, B.L., Desouza, C.A., 2011. Endothelin-1 vasoconstriction and the age-related decline in endothelium-dependent vasodilatation in men. Clinical Science 120, 485–491. https://doi.org/10.1042/CS20100475

White, J.G., 1998. Use of the electron microscope for diagnosis of platelet disorders. Semin Thromb Hemost 24, 163–168. https://doi.org/10.1055/s-2007-995836

Whorton, A.R., Willis, C.E., Kent, R.S., Young, S.L., 1984. The role of calcium in the regulation of prostacyclin synthesis by porcine aortic endothelial cells. Lipids 19, 17–24. https://doi.org/10.1007/BF02534603

Wickham, K.A., Spriet, L.L., 2023. Food for Thought: Physiological Considerations for Nutritional Ergogenic Efficacy. Scandinavian Journal of Medicine & Science in Sports. *Ahead of Print*. https://doi.org/10.1111/sms.14307

Wiik, A., Hellsten, Y., Berthelson, P., Lundholm, L., Fischer, H., Jansson, E., 2009. Activation of estrogen response elements is mediated both via estrogen and muscle contractions in rat skeletal muscle myotubes. Am J Physiol Cell Physiol 296, C215-220. https://doi.org/10.1152/ajpcell.00148.2008

Wilkerson, W.R., Sane, D.C., 2002. Aging and thrombosis. Semin Thromb Hemost 28, 555–568. https://doi.org/10.1055/s-2002-36700

Willhite, L.A., O'Connell, M.B., 2001. Urogenital Atrophy: Prevention and Treatment. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 21, 464–480. https://doi.org/10.1592/phco.21.5.464.34486

World Health Organization, 2022. Cardiovascular diseases [WWW Document]. URL https://www.who.int/health-topics/cardiovascular-diseases (accessed 10.12.22).

Woulfe, D., Yang, J., Brass, L., 2001. ADP and platelets: the end of the beginning. J Clin Invest 107, 1503–1505.

Xia, H., Dufour, C.R., Giguère, V., 2019. ERRα as a Bridge Between Transcription and Function: Role in Liver Metabolism and Disease. Frontiers in Endocrinology 10.

Xu, X., Wang, B., Ren, C., Hu, J., Greenberg, D.A., Chen, T., Xie, L., Jin, K., 2017. Age-related Impairment of Vascular Structure and Functions. Aging Dis 8, 590–610. https://doi.org/10.14336/AD.2017.0430

Xu, Y., Shen, Y., Chen, D., Zhao, P., Jiang, J., 2021. Efficacy and Safety of Dual Antiplatelet Therapy in Patients Undergoing Coronary Stent Implantation: A Systematic Review and Network Meta-Analysis. Journal of Interventional Cardiology 2021, e9934535. https://doi.org/10.1155/2021/9934535

Yaffe, K., Sawaya, G., Lieberburg, I., Grady, D., 1998. Estrogen Therapy in Postmenopausal WomenEffects on Cognitive Function and Dementia. JAMA 279, 688–695. https://doi.org/10.1001/jama.279.9.688

Yousefzadeh, M., Henpita, C., Vyas, R., Soto-Palma, C., Robbins, P., Niedernhofer, L., 2021. DNA damage-how and why we age? Elife 10, e62852. https://doi.org/10.7554/eLife.62852

Yuan, S.Y., Rigor, R.R., 2010. Structure and Function of Exchange Microvessels, Regulation of Endothelial Barrier Function. Morgan & Claypool Life Sciences.

Yun, S.-H., Sim, E.-H., Goh, R.-Y., Park, J.-I., Han, J.-Y., 2016. Platelet Activation: The Mechanisms and Potential Biomarkers. BioMed Research International 2016, e9060143. https://doi.org/10.1155/2016/9060143

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J., Lisheng, L., INTERHEART Study Investigators, 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 364, 937–952. https://doi.org/10.1016/S0140-6736(04)17018-9

Zhong, P.-Y., Shang, Y.-S., Bai, N., Ma, Y., Niu, Y., Wang, Z.-L., 2021. Efficacy and Safety of Very Short-Term Dual Antiplatelet Therapy After Drug-Eluting Stents Implantation for Acute Coronary Syndrome: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. Front Cardiovasc Med 8, 660360. https://doi.org/10.3389/fcvm.2021.660360

CHAPTER 9 APPENDICES

9.1 Platelet Reactivity Optimization Summary

9.1.1 Centrifugation

- We tried two different centrifugation durations (10 min vs. 20 min) for obtaining platelet-rich plasma. Although we obtained a greater volume of greater platelet-rich plasma with 20 min centrifugation (~1000 µL more platelet-rich plasma from 9 mL whole blood), we opted for 10 min centrifugation to minimize platelet activation by prolonged centrifugation, as our assay measures platelet activation and aggregation to known concentrations of agonists.

9.1.2 Collagen Platelet Reactivity

- We attempted the platelet reactivity assay with 5% isotonic glucose (pH 2.7) prepared in ddH_2O as well as 10 mM isotonic glucose in 1x PBS. Results were better with 10 mM isotonic glucose in 1x PBS (better range and reproducibility).
- We started with collagen concentrations ranging from 0.01 to 10 µg/mL with a 4-fold dilution, however we were not capturing the high-end of the platelet reactivity curve.
- We switched to concentrations ranging from 0.02 to 16 µg/mL with a 4-fold dilution.

9.1.3 Adenosine 5'Diphosphate (ADP) Platelet Reactivity

- We started with ADP concentrations ranging from 0.02 to 40 µM with a 3.25-fold dilution, however we were not capturing the high-end of the platelet reactivity curve.
- We switched to concentrations ranging from 0.08 to 80 µM with a 4-fold dilution.

9.1.4 Epinephrine Platelet Reactivity

- We started with epinephrine concentrations ranging from 0.04 to 40 µM with a 4-fold dilution, however we were not capturing the low- or high-ends of the platelet reactivity curves.
- We switched to epinephrine concentrations ranging from 0.001 to 40 µM with a 6-fold dilution.

9.1.5 Arachidonic Acid Platelet Reactivity

- We attempted to assess platelet reactivity to arachidonic acid, as a measure of the effectiveness of aspirin treatment in the dual anti-platelet therapy (DAPT) study. However, we couldn't achieve greater than ~5% platelet reactivity utilizing concentrations ranging from 0.06 to 1 mM, 0.05 to 5 mM, and 0.05 to 11.1 mM. We decided against utilizing this agonist.

9.2 Platelet Isolation Optimization Summary

9.2.1 Utilization of Density Gradient

- Initially, we attempted to isolate platelets by obtaining platelet-rich plasma, which was then pipetted on top of a density gradient (OptiPrep Density Gradient Medium, D1556, Sigma-Aldrich, USA) in a ratio of 1.0 mL platelet-rich plasma to 0.75 mL density gradient. This was centrifuged at 450g for 15 min to ensure the isolation of pure platelets. Then the supernatant was collected, a full hematological count including a platelet count was then obtained, and then centrifuged for 15 min at 1500g to form a platelet pellet. The supernatant was then pipetted away and the pellet was resuspended in 1x DPBS. Another full hematological count including a platelet count was then obtained.
- Although this protocol yielded pure platelets, the platelet counts were very low at both the density gradient step as well as the platelet pelleting step, suggesting the platelets were being lost in the density gradient step. In addition, the platelets were sticking to the bottom of the collection tubes, suggesting they were becoming activated during the pelleting step.
- We attempted to optimize the protocol by obtaining the platelet-rich plasma at different speeds (200g vs. 500g vs. 900g) as well as forming the platelet pellet at different centrifugation speeds (200g vs. 500g vs. 900 g). All attempts resulted in poor platelet yields.

9.2.2 Switching to Prostaglandin E_1 and Resuspension Buffer

- To mitigate the issues observed with the density gradient protocol, we decided to obtain platelet-rich plasma, which was then transferred to an Eppendorf tube and treated with 1 µL of prostaglandin E_1 per 100 µL platelet-rich plasma. This reversibly inhibited platelet activation for ~20 minutes. The platelet-rich plasma was then spun for 5 min at 1500g to form a platelet pellet. The platelet pellet was then resuspended in 400 µL of a physiologically relevant resuspension buffer, and a full hematological count including a platelet count was then obtained.

- The remaining issue was that when the platelets were loaded for western blot analysis, there was smearing on the stain-free images, suggesting that some residual serum or plasma components were present in the isolated platelets.

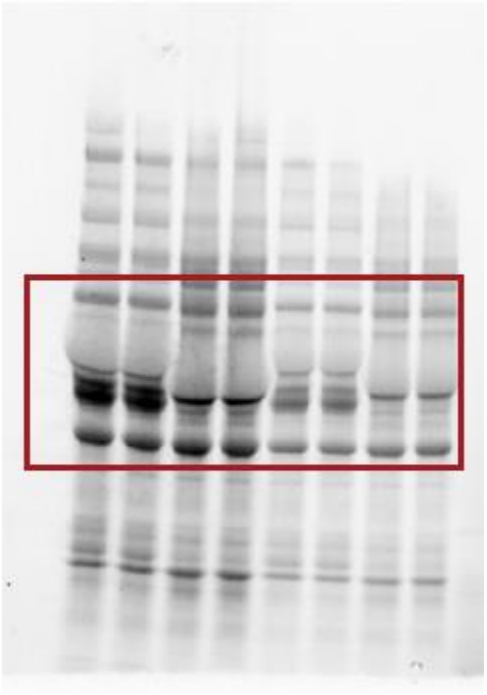

Figure 9-1: Smearing on stain-free image suggesting residual plasma constituents.

9.2.3 Selection of the Lysis Buffer

- In our platelet western blot pilot, we prepared isolated platelets in two different concentrations to get an estimate of the number of platelets required: 0.25 x 10^9 or 0.5 x 10^9 platelets per well were loaded.
- In addition, we attempted preparing the platelets for western blotting in three different commonly used lysis buffers: Triton X-100 (T8787, Sigma-Aldrich), mtDNA lysis buffer (100 mM NaCl, 10 mM Tris; pH 8.0, 25 mM EDTA; pH 8.0, 0.25% SDS, H_2O, proteinase K), and MSD Tris Lysis Buffer (R60TX-2, MSD).
- From this pilot run, we determined that both 0.25 and 0.5 x 10^9 platelets per well was sufficient. In addition, we determined that both Triton X-100 and MSD Tris Lysis Buffer were effective for western blotting. Interestingly, we did not obtain any signal when using mtDNA lysis buffer, either on the stain-free image or western blot. We proceeded with Triton X-100, as it was much less expensive.

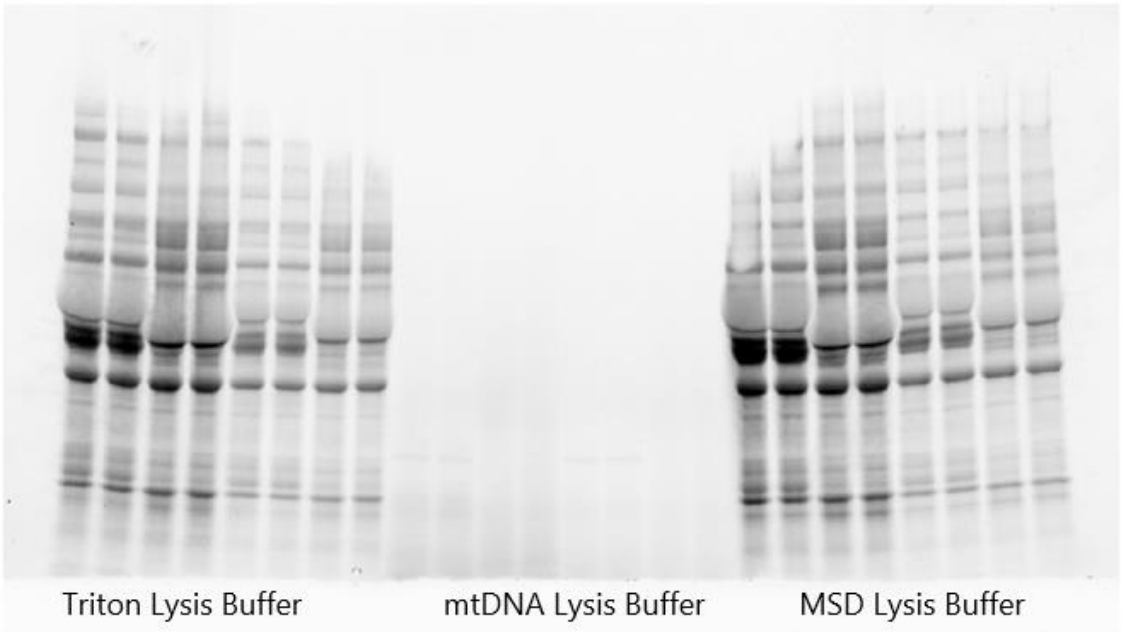

Figure 9-2: Selection of a lysis buffer.

Stain-free image showing that with mtDNA lysis buffer the signal is completely lost from all loaded protein. Alternatively, the signal from Triton X100 and MSD lysis buffer are of similar, good quality.

9.2.4 Addition of a Wash Buffer Step

- To further remove the residual plasma constituents, we added a wash step after the formation of the platelet pellet, where an equal volume of a physiologically relevant wash buffer to the original volume of platelet-rich plasma was added to the platelet pellet. The Eppendorf tube was gently inverted several times before an additional centrifugation step of 5 min at 1500g. Then, the wash buffer supernatant was removed, and the pellet was resuspended in 400 µL of resuspension buffer. A full hematological count including a platelet count was then obtained.
- The stain-free images for western blot analysis no longer had smearing, suggesting that all or almost all of the plasma constituents were successfully removed from by the added wash buffer step.

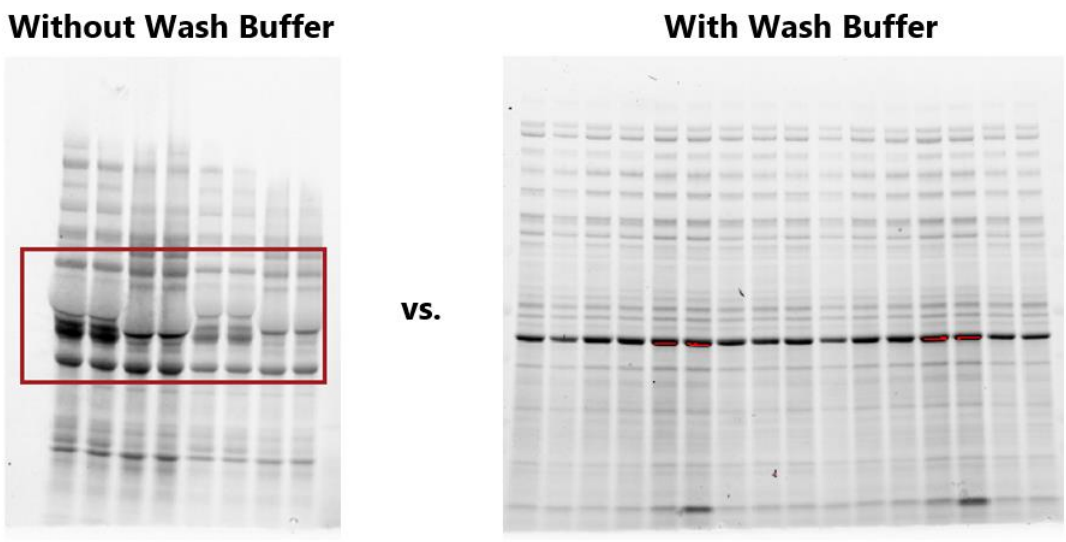

Figure 9-3: Addition of a wash buffer step

Utilization of a wash buffer completely removed the smearing on the stain-free image, suggesting that the residual plasma constituents were removed by this process.

9.2.5 Determining the Number of Platelets to Load in Each Well

- To determine the number of platelets that needed to be loaded for optimal western blot analysis, an optimization western blot run was performed. This entailed utilizing the platelet isolates with the highest platelet count (e.g., ~5000 x 10^3 platelets·µL^{-1}) and preparing various fold dilutions (e.g., 1:5, 1:10, 1:15) to ensure all the ranges of platelet counts were captured.
- Once the appropriate dilution was determined, the total number of platelets loaded was calculated, then using ratios the dilution factor for each individual sample was determined to ensure the total number of platelets loaded was approximately the same for each sample.

Participant ID	PRE Platelet Count	PRE Platelet Count (plt/µL)	Dilution	POST Platelet Count	POST Platelet Count (plt/µL)	Dilution
BEL32	76,400,000	95500	Full	71,200,000	89000	Full
TN34	1,144,800,000	1431000	1:5	1,146,400,000	1433000	1:5
MC24	1,206,400,000	1508000	1:5	1,877,200,000	2346500	1:10
PJ27	477,200,000	596500	Full	201,600,000	252000	Full
LLL25	1,557,200,000	1946500	1:7.5	864,000,000	1080000	1:4
MML36	1,432,400,000	1790500	1:7.5	1,984,800,000	2481000	1:10
LMA19	1,733,200,000	2166500	1:10	1,620,800,000	2026000	1:10
EV26	1,800,400,000	2250500	1:10	38,000,000	47500	Full
JS23	1,848,800,000	2311000	1:10	2,038,000,000	2547500	1:10
EWP22	1,237,200,000	1546500	1:5	1,004,800,000	1256000	1:5

Figure 9-4: Determining the appropriate dilution of isolated platelets for western blot analysis.

9.2.6 Consistency in Platelet Procedures

	Pre-Training	Post-Training
Blood Draw to Centrifugation (min)	32 ± 2	22 ± 5
Platelet Reactivity Assay (min)	55 ± 2	58 ± 1
Prostacyclin Sensitivity Assay (min)	73 ± 3	74 ± 2

Figure 9-5: Elapsed time for various steps of the platelet reactivity assay protocols utilized in Study I.

Note: Time for the platelet reactivity and prostacyclin sensitivity assays was recorded from the onset of centrifugation to the time of reading the 96-well plate.

	Pre-Training	Post-Training
Blood Draw to Centrifugation (min)	133 ± 78	129 ± 54
Platelet Count ($x10^3$; platelet·μL^{-1})	2689 ± 1571	2691 ± 1844
Purity of Isolated Platelets (%)	95 ± 3	92 ± 6

Figure 9-6: Elapsed time and platelet quality control measures from the platelet isolation protocol utilized in Study I.

- In Studies I and II, blood sampling was performed on two separate days to minimize the volume of blood drawn each day (i.e., ~60 mL each day vs. ~120 mL on one day). This was done to minimize the impact of blood removal on the $\dot{V}O_{2max}$ test results and to minimize the potential for participants to feel faint, particularly in a fasted state.

9.3 Exercise Training Intensity Verification

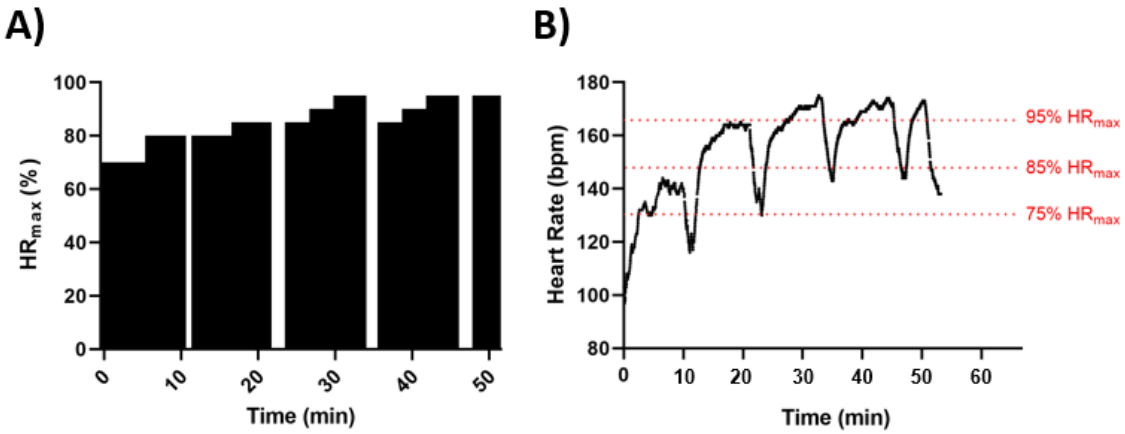

Figure 9-7: A) Overview of the spinning training intervention, where the rest periods involved low-intensity self-selected pace cycling (i.e., no resistance). B) representative continuous heart rate trace throughout the spinning session.

- The floorball training was typically performed as small-sided games (e.g., 3 vs. 3) with 4 min of high-intensity effort followed by 2 min of rest, repeated for 60 min.
- When necessary, the floorball training was modified according to the number of participants in each session.
- Continuous heart rate monitoring includes warm-up, cool-down, and rest periods.

9.4 Ethics certificate for Chapter 4 and 6

Region Hovedstaden

Center for Regional Udvikling

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Journal-nr.: H-20037633

Dato: 20-01-2021

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H-20037633 - Effekt af holdspil på kardiovaskulær sundhed hos ældre mænd og kvinder.

Endelig godkendelse.

Afgørelsen er truffet efter lovbekendtgørelse nr. 1338 af 1. september 2020 - lov om videnskabsetisk behandling af sundhedsvidenskabelige forskningsprojekter og sundhedsdatavidenskabelige forskningsprojekter.

Jeg bekræfter modtagelsen af mail af 6. januar 2021, som svar på afgørelse af 20. december 2020, hvori der opstilledes betingelser for godkendelsen af projektet.

Betingelserne for godkendelsen anses for opfyldt. Projektet er dermed endeligt godkendt.

Godkendelsen gælder til den 1. maj 2025 og omfatter følgende dokumenter:

- Forsøgsprotokol, version 3, af 5. januar 2021
- Deltagerinformation A, version 3, af 5. januar 2021
- Resumé af deltagerinformation A, version 3, af 5. januar 2021
- Deltagerinformation B, version 3, af 5. januar 2021
- Resumé af deltagerinformation B, version 3, af 5. januar 2021
- Informeret samtykkeerklæring, modtaget 24. november 2020
- Rekrutteringstekster version 2, af 24. november 2020
- Tekst til hjemmeside, version 2, af 22. november 2020
- Information til foreninger, modtaget 24. november 2020

Godkendelsen gælder for de anmeldte forsøgssteder og den anmeldte forsøgsansvarlige i Danmark.

Komiteen er ikke ressortmyndighed for regelsættet om databeskyttelse. Komiteen forudsætter at projektet gennemføres i overensstemmelse med databeskyttelsesforordningen og databeskyttelsesloven.

Iværksættelse af projektet i strid med godkendelsen kan straffes med bøde eller fængsel, jf. komitélovens § 41.

Ændringer

Foretages der væsentlige ændringer i protokolmaterialet under gennemførelsen af projektet, skal disse anmeldes til komiteen i form af tillægsprotokoller. Ændringerne må først iværksættes efter godkendelse fra komiteen, jf. komitélovens § 27, stk. 1.

Anmeldelse af tillægsprotokoller skal ske elektronisk på www.drvk.dk/anmeldelse med det allerede tildelte anmeldelsesnummer og adgangskode.

Væsentlige ændringer er bl.a. ændringer, der kan få betydning for forsøgspersonernes sikkerhed, fortolkning af den videnskabelige dokumentation, som projektet bygger på samt gennemførelsen eller ledelsen af projektet. Det kan fx være ændringer i in- og eksklusionskriterier, forsøgsdesign, antal forsøgspersoner, forsøgsprocedurer, behandlingsvarighed, effektparametre, ændringer om de forsøgsansvarlige eller forsøgssteder samt indholdsmæssige ændringer i det skriftlige informationsmateriale til forsøgspersonerne.

Hvor nye oplysninger betyder, at forskeren overvejer at ændre proceduren eller stoppe forsøget, skal komiteen orienteres om det.

Bivirkninger og hændelser

Løbende indberetning

Komiteen skal omgående underrettes, hvis der under projektet optræder formodet alvorlige, uventede bivirkninger eller alvorlige hændelser, jf. komitélovens § 30, stk. 1. Indberetningen skal ledsages af kommentarer om eventuelle konsekvenser for forsøget. Det er kun bivirkninger og hændelser forekommet i Danmark, der skal indberettes. Underretning skal ske senest 7 dage efter, at sponsor eller den forsøgsansvarlige har fået kendskab til tilfældet.

Ved indberetning kan anvendes et skema, der findes på www.nvk.dk. Skemaet med bilag kan indsendes elektronisk ved anvendelse af digital signatur.

Årlig indberetning

Én gang årligt i hele forsøgsperioden skal komiteen have tilsendt en liste over alle formodet alvorlige (ventede og uventede) bivirkninger og alvorlige hændelser, som er indtruffet i forsøgsperioden sammen med en rapport om forsøgspersonernes sikkerhed, jf. komitélovens § 30, stk. 2.

Materialet skal være på dansk eller engelsk.

Ved indberetning skal anvendes et skema, der findes på www.nvk.dk. Skemaet med bilag kan indsendes elektronisk ved anvendelse af digital signatur.

Afslutning

Den forsøgsansvarlige og en evt. sponsor skal senest 90 dage efter afslutningen af projektet underrette komiteen herom, jf. komitélovens § 31, stk. 1. Projektet regnes som afsluttet, når sidste forsøgsperson er afsluttet.

Afbrydes projektet tidligere end planlagt, skal en begrundelse herfor sendes til komiteen senest 15 dage efter, at beslutningen er truffet, jf. komitélovens § 31, stk. 2.

Hvis projektet ikke påbegyndes, skal dette samt årsagen hertil meddeles komiteen.

Komiteen beder om kopi af den afsluttende forskningsrapport eller publikation, jf. komitelovens § 28, stk. 2. Vi skal i den forbindelse gøre opmærksom på, at der er pligt til at offentliggøre både negative, positive og inkonklusive forsøgsresultater, jf. komitélovens § 20, stk. 1, nr. 8.

Pligten til at indberette afsluttende forsøg og rapport påhviler forsøgsansvarlig og en evt. sponsor i forening.

Tilsyn

Komiteen fører tilsyn med, at projektet udføres i overensstemmelse med godkendelsen, jf. komitélovens § § 28 og 29.

Underskrift på samtykkeerklæringen

Komiteen gør opmærksom på, at forsøgsansvarlig kan delegere sin pligt til at underskrive samtykkeerklæringen til den person, der holder den mundtlige informationssamtale. Der skal i så fald være en skriftlig delegation hertil på forsøgssitet.

Med venlig hilsen

Anne Marquardt
Cand. Jur.

Kopi sendt til:

- Line Nørregaard Olsen

9.5 Ethics certificate for Chapter 7

Regionshuset
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Godkendelse

Projekt: Effekt af fermenteret rødkløver på muskelstyrke og muskelproteinsignalering – et randomiseret kontrolleret overkrydningsforsøg.

De Videnskabsetiske Komitéer for Region Midtjylland, Komité I, har behandlet projektet på møde den 8. oktober 2019, og du har efterfølgende indsendt revideret materiale. Komitéen har på den baggrund truffet følgende afgørelse.

Afgørelse:

Projektet godkendes i henhold til lovbekendtgørelse nr. 1083 af 15. september 2017 om bekendtgørelse af lov om videnskabsetisk behandling af sundhedsvidenskabelige forskningsprojekter.

Godkendelsen gælder for de anmeldte forsøgssteder, den anmeldte forsøgsansvarlige i Danmark samt for den angivne forsøgsperiode.

Godkendelsen gælder til den 1. juli 2025 og omfatter følgende dokumenter:

- Forsøgsprotokol, version 2, dateret 22. oktober 2019, fremsendt med mail af 24. oktober 2019.
- Opslag med henblik på rekruttering af forsøgspersoner, version og dato ikke angivet, fil navngivet version1_12092019, fremsendt med mail af 12. september 2019.
- Deltagerinformation, version 2, dateret 22. oktober 2019, fremsendt med mail af 24. oktober 2019.
- Samtykkeerklæring (S4), version og dato ikke angivet, fil navngivet version1_12092019, fremsendt med mail af 12. september 2019.
- Spørgeskema fremsendt med mail af 12. september 2019 er godkendt til udlevering.

Det bemærkes, at komitéen ikke er ressortmyndighed vedr. regelsættet om databeskyttelse, og at komiteen forudsætter, at projektets indhold vedr. dette er i overensstemmelse med Europa-Parlamentets og Rådets forordning nr. 2016/679 af 27. april 2016 om beskyttelse af fysiske personer i forbindelse med behandling af personoplysninger og om fri udveksling af sådanne oplysninger og databeskyttelsesloven.

Iværksættelse af projektet i strid med godkendelsen kan straffes med bøde eller fængsel, jf. komitélovens § 41.

Ændringer:

Foretages der væsentlige ændringer i protokolmaterialet under gennemførelsen af projektet, skal disse anmeldes til komitéen i form af tillægsprotokoller. Ændringerne må først iværksættes efter godkendelse fra komitéen, jf. komitélovens § 27, stk. 1.

Anmeldelse af tillægsprotokoller skal ske elektronisk på *www.drvk.dk* med det allerede tildelte anmeldelsesnummer og adgangskode.

Væsentlige ændringer er bl.a. ændringer, der kan få betydning for forsøgspersonernes sikkerhed, fortolkning af den videnskabelige dokumentation, som projektet bygger på samt gennemførelsen eller ledelsen af projektet. Det kan fx være ændringer i in- og eksklusionskriterier, forsøgsdesign, antal forsøgspersoner, projektforlængelse, forsøgsprocedurer, behandlingsvarighed, effektparametre, ændringer om de forsøgsansvarlige eller forsøgssteder samt indholdsmæssige ændringer i det skriftlige informationsmateriale til forsøgspersonerne.

Hvor nye oplysninger betyder, at forskeren overvejer at ændre proceduren eller stoppe forsøget, skal komitéen orienteres om det.

Bivirkninger og hændelser:

Løbende indberetning
Komitéen skal omgående underrettes, hvis der under projektet optræder formodet alvorlige, uventede bivirkninger eller alvorlige hændelser, jf. komitélovens § 30, stk. 1.

Indberetningen skal ledsages af kommentarer om eventuelle konsekvenser for forsøget. Det er kun bivirkninger og hændelser forekommet i Danmark, der skal indberettes. Underretning skal ske senest 7 dage efter, at sponsor eller den forsøgsansvarlige har fået kendskab til tilfældet.

Ved indberetning kan anvendes et skema, der findes på *www.nvk.dk*. Skemaet med evt. bilag skal indsendes elektronisk i pdf-format til *komite@rm.dk*.

Årlig indberetning
Én gang årligt i hele forsøgsperioden skal komitéen have tilsendt en liste over alle formodet alvorlige (ventede og uventede) bivirkninger og alvorlige hændelser, som er indtruffet i forsøgsperioden sammen med en rapport om forsøgspersonernes sikkerhed, jf. komitélovens § 30, stk. 2. Har der ikke været alvorlige bivirkninger og hændelser skal dette ligeledes indberettes.

Ved indberetning kan anvendes et skema, der findes på *www.nvk.dk*. Skemaet med evt. bilag skal indsendes elektronisk i pdf-format til *komite@rm.dk*.

Afslutning:

Den forsøgsansvarlige skal senest 90 dage efter afslutningen af projektet underrette komitéen herom, jf. komitélovens § 31, stk. 1. Projektet regnes som afsluttet, når indsamling af data er afsluttet.

Afbrydes projektet tidligere end planlagt, skal en begrundelse herfor sendes til komitéen senest 15 dage efter, at beslutningen er truffet, jf. komitélovens § 31, stk. 2.

Hvis projektet ikke påbegyndes, skal dette samt årsagen hertil meddeles komitéen.

Komitéen beder om kopi af den afsluttende forskningsrapport eller publikation, jf. komitélovens § 28, stk. 2. Vi skal i den forbindelse gøre opmærksom på, at der er pligt til at offentliggøre både negative, positive og inkonklusive forsøgsresultater, jf. komitélovens § 20, stk. 1, nr. 8.

Tilsyn:

Komitéen fører tilsyn med, at projektet udføres i overensstemmelse med godkendelsen, jf. komitélovens § 28 og § 29.

Følgende komitémedlemmer deltog i mødebehandlingen:

Fagpersoner

- Helene Nørrelund (formand)
- Nete Hornung
- Niels Henrik Vinther Krarup
- René Frydensberg Andersen
- Sanne Fisker

Lægpersoner

- Peder Meyhoff (næstformand)
- Annette Roed
- Louise Ahle
- Ole Kamp

Venlig hilsen

Anne-Marie Eybye
Sekretær