

## Review Article

# The effect of female sex hormone supplementation on tendon in pre and postmenopausal women: A systematic review

C. Ganderton<sup>1</sup>, A. Semciw<sup>1,2</sup>, J. Cook<sup>1</sup>, T. Pizzari<sup>1</sup><sup>1</sup> Faculty of Health Science, School of Allied Health, Department of Physiotherapy, La Trobe University, Australia;<sup>2</sup> School of Health of Rehabilitation Sciences, University of Queensland, Australia**Abstract**

**Objectives:** Oestrogen deprivation has been shown to have a negative effect on connective tissue and its turnover. A link may exist between supplemental oestrogen, preservation of tendon collagen and less tendon abnormality. The aim was to determine the effects of female sex hormone supplementation (FSHS) on tendon. **Methods:** A systematic search of nine key health databases; Medline, CINAHL, EMBASE, SPORTDiscus, AUSPORT and AMI, Cochrane Library, SafetyLit and PEDro was completed (to Feb 24, 2016). The search yielded 6378 records using terms relating to *hormone* (oestrogen, estrogen, hormone replacement therapy, HRT, estrogen therapy, oestrogen therapy, oral contraceptive pill) and *tendon*. Quality assessment, data extraction and data synthesis of included papers was undertaken. **Results:** Low level of evidence for all outcomes; no positive or negative link between FSHS and molecular, mechanical and morphological tendon response outcomes, and the addition of exercise to FSHS, had minimal effects on tendon CSA. **Conclusions:** The effect of oestrogen supplementation on tendon is contradictory and inconsistent. This review suggests there is a need for further studies to understand the effects of FSHS on tendon tissue at a mechanical, morphological and molecular level.

**Keywords:** Hormone Replacement Therapy, Oestrogen, Oral Contraceptive Pill, Women, Tendon

**Introduction**

Tendinopathy is the clinical diagnosis given to a tendon causing pain and dysfunction. Tendinopathy affects active and inactive populations, from adolescents through to the elderly<sup>1</sup>, and can impact on quality of life<sup>2</sup> and the ability to participate in pain-free activity. Rehabilitation is long, tedious and often expensive with many patients seeking multiple therapies, secondary to ineffective treatment regimens<sup>1</sup>.

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Tendinopathy is usually associated with pathology within the tendon, although, pathology can be present for years<sup>3</sup>, without causing symptoms<sup>4,5</sup>. Tendon pathology is characterised by a change in cell function, increase in ground substance, disorganisation of collagen and neovascularisation<sup>6,7</sup> evident on ultrasound or magnetic resonance imaging.

A number of risk factors for tendinopathy are associated with levels of circulating female sex hormones. Advancing age, female gender, body composition, and genetic make-up can all influence circulating sex hormone levels<sup>1,7-9</sup> and may predispose to tendon pain. As oestrogen levels decline after menopause, collagen production can decline and tendons may become thinner<sup>10</sup>, rates of tendon pathology and the likelihood of tendon rupture increases<sup>11,12</sup>. Where oestrogen levels are higher in pre-menopausal females, the likelihood of developing lower limb tendinopathy is lower than that of age matched men<sup>4</sup>.

Body shape and fat distribution have also been associated with changes in oestrogen levels<sup>13</sup> and tendon pathology<sup>14,15</sup>. Those with an android compared to a gynoid body shape are more likely to develop tendon pathology<sup>16</sup>. There is emerg-

ing evidence to suggest that supplemental oestrogen directly affects fibroblast proliferation<sup>17,18</sup> and collagen synthesis<sup>19,20</sup> and may change the susceptibility to tendinopathy. Thus, investigation of the impact of female sex hormones on tendon molecular, mechanical and morphological outcomes, and CSA area is therefore warranted.

Topical, subcutaneous and, more commonly, oral oestrogen is prescribed in both pre and post-menopausal populations, and the effect on connective tissue structures has been widely researched. It is known that supplemental oestrogen is beneficial for increasing skeletal muscle strength<sup>21</sup>, reducing fractures<sup>22</sup>, preserving bone mass<sup>23</sup> and preventing a decline in the collagen content of skin<sup>24,25</sup>. The effect of oestrogen on collagen turnover in connective tissue varies, with studies finding an inhibiting effect<sup>17,26</sup>, no effect<sup>27</sup> and a stimulating effect<sup>28</sup>. The reported higher rate of anterior cruciate ligament injury in women compared to men, has been attributed to the rhythmic variation of fluctuating oestrogen levels, but this remains both equivocal and controversial in the literature<sup>29,30</sup>.

Little is known about the effects of female sex hormones on tendon and it is hypothesised that the effects could be a direct and/or indirect. Tendon cells contain hormone-specific receptors and evidence suggests that tendon may be responsive to oestrogen<sup>31</sup>, directly effecting type 1 collagen upregulation and turnover<sup>19</sup>. Animal studies have also linked progesterone and high dose oestrogen with the upregulation of relaxin receptor isoforms (RXFP1 and RXFP2) in tendon<sup>32</sup>. Relaxin has an effect on the extracellular matrix of tendon tissue. While animal and basic science studies have shown structural and molecular changes in response to sex hormones, it is unclear how they affect the mechanical properties of the tendon, or in turn the propensity to develop tendon pathology or pain.

Sex hormones have been reported to alter mechanical (tendon stress: failure point of the tendon measured in megapascals (MPa); strain:  $\Delta\text{length}/\text{length}$ ; stiffness:  $\Delta\text{force}/\text{length}$ ); Young's Modulus:  $\Delta\text{stress}/\Delta\text{strain}$ )<sup>33</sup>, molecular (tendon collagen synthesis)<sup>19,34</sup> and morphological (tendon CSA)<sup>1</sup> properties of human tendon. Despite the research to date, the direct and indirect effects of female sex hormone supplementation on human tendon tissue are not well understood, and it is unknown as to whether increasing or decreasing these factors are beneficial<sup>19</sup>. It is plausible that supplemental oestrogen (hormone replacement therapy: HRT) may be beneficial for preserving tendon collagen<sup>10</sup> and reduced tendon abnormality<sup>1</sup>.

## Objective

The primary aim of this review was to evaluate the effect of female sex hormone supplementation (FSHS) on tendon tissue in pre and post-menopausal women. The secondary objective was to assess, within the included studies, whether the addition of exercise to FSHS alters the mechanical, morphological and molecular response of tendon.

## Data sources

### Search methods and study selection

Database searches were conducted by one reviewer (CG) accessing Medline (1946 to Feb 24, 2016), CINAHL (1984 to Feb 24, 2016), EMBASE (1988 to Feb 24, 2016), all Cochrane Library databases (to Feb 24, 2016), PEDro (to Feb 24, 2016), SPORTDiscus (1937 to Feb 24, 2016), AUSPORT (1970 to Feb 24, 2016), SafetyLit (to Feb 24, 2016) and AMI (1970 to Feb 24, 2016). A subject heading and key word search was performed using two main concepts: *hormone* (oestrogen, estrogen, hormone replacement therapy, HRT, estrogen therapy, oestrogen therapy, oral contraceptive pill) and *tendon*. All references were imported into Endnote version X6 (Thomson Reuters, New York, NY, USA) and duplicate articles were deleted by one reviewer (CG). Three reviewers (CG - all, TP - L-Z and AS - A-K) independently applied the selection criteria to the titles and abstracts of the remaining studies and excluded studies that did not clearly meet the criteria. There were no disagreements between independent reviewers during this process. Full texts were obtained where the title and abstract appeared to meet the criteria or did not provide sufficient information to determine eligibility. The criteria were reapplied to all retrieved full text articles by two reviewers in consultation (CG, TP).

Citation tracking of included studies was performed using Google Scholar. A manual check of references in key articles was also performed to ensure that no relevant articles had been missed in the initial search.

## Study selection

### Eligibility criteria

**Participants:** Studies assessing pre and/or post-menopausal women were included. Studies were required to document hormonal status.

**Types of Intervention:** A comparison of FSHS versus no hormone supplementation (control group) was required for studies to be included in this review. Female sex hormone supplementation interventions of any frequency and duration were included in the study. Acceptable FSHS interventions included the oral contraceptive pill, transdermal oestrogen and HRT/oestradiol therapy.

### Types of outcome measures

Studies must have assessed one or more of: the molecular response, morphological response or mechanical response of tendon to FSHS. Outcomes must have been evaluated in both the intervention (FSHS) group and control group. Additionally, all outcomes must have been measured at a resting state but could additionally be measured during or post-exercise. Any non-tendon outcomes, such as those relating muscle and bone, were excluded from the review. Where the same outcome was reported twice on the same cohort<sup>20,35</sup>, only the study with the most complete dataset was included<sup>20</sup>.

### Study characteristics

Only full text articles published in peer-reviewed journals were included and no restrictions on language were applied to the search. Studies on the prevalence of tendon abnormality associated with FSHS were included. Case studies, case series and opinion articles were excluded due to the greater potential for bias in these types of studies<sup>36</sup>.

### Data extraction

#### Appraisal of included studies

Due to the varying susceptibility to bias in non-randomised studies, all included papers were assessed for methodological quality by one reviewer (CG) using an observational study assessment form. This form was adapted from a quality tool employed by Siegfried et al<sup>37</sup>. The Non-Randomised Studies Methods Group of The Cochrane Collaboration has commended this quality assessment tool<sup>38</sup>. This tool was chosen as it evaluates external and internal validity (performance bias, detection bias and selection bias). Furthermore, it itemises and displays each aspect of quality assessment in its raw form for readers. Specific criteria were created to evaluate bias and confounding factors in study design – level of hormone administered, duration of HRT or oral contraceptive pill (OCP) usage prior to the study, testing procedure, baseline participant fitness/activity levels, present co-morbidities, participant weight/body mass index, the use of separate exercise and non-exercise groups to assess the impact of exercise with FSHS, and randomisation of intervention. Unlike many quality assessment tools, a summary score is not used; rather, the reader is able to evaluate the studies by each category and hence study quality validity is not compromised<sup>37</sup>.

#### Quality of evidence

The level of evidence for each review outcome was evaluated based on methods first described by vanTulder<sup>39</sup> and modified by Yusuf et al.<sup>40</sup> due to heterogeneity of the included studies. There were five levels of evidence, with strong evidence indicating consistent findings among high quality studies, moderate evidence indicating generally consistent findings from at least one high quality study and multiple low quality studies, limited evidence indicating generally consistent results from two or more low quality studies, very limited evidence indicating results from one low quality study and conflicting evidence indicating inconsistent findings among multiple low or high quality statistically heterogeneous studies. This synthesis places weight on the quality of studies and consistency of results for each outcome.

This review defined a 'high quality' study was as one that randomised participants to the intervention, and fulfilled nine of the eleven other categories on the quality tool employed (Table II). A 'low quality' study was defined as one that did not randomise participants to the intervention and fulfilled less than nine of the eleven other categories.

### Data extraction methods

A customised data extraction form based on the recommendations of the National Randomised Studies and Methods Group (NRSMG)<sup>41</sup> and the Transparent Reporting of Evaluations with Non-randomised Designs (TREND) statement<sup>42</sup>, was used to guide data extraction for non-randomised studies. Information obtained included (but was not limited to) study inclusion and exclusion criteria, details of methodology, outcome measures, confounding variables and any statistical analyses. Where studies incorporated a non-exercise and active/exercise or post-exercise condition, both sets of data were included in the review.

#### Data synthesis

Review Manager (RevMan Version 5.2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012) was used to create forest plots and calculate effect sizes via standardised mean differences and 95% confidence intervals (CI) for study outcomes. A small number of studies investigating each outcome meant that it was inappropriate to combine most of the data into a meta-analysis<sup>41,43</sup>. A meta-analysis was performed on outcomes where studies were clinically and statistically homogenous. Statistical homogeneity was determined by the  $I^2$  value<sup>44</sup>, where a value of 0% indicates no observed heterogeneity, less than 25% is considered to have low levels and a value of 100% indicates a completely heterogeneous sample. For ease of analysis, 'non-exercise leg' data were grouped with 'inactive' and 'contralateral leg' participant data, and 'exercise leg' data were grouped with 'active' and 'jumping leg' participant data. Odds ratios (OR) were also calculated to determine the likelihood of tendon abnormality, where ultrasonographic abnormal-

Figure 1. Flowchart of the studies included in the review.

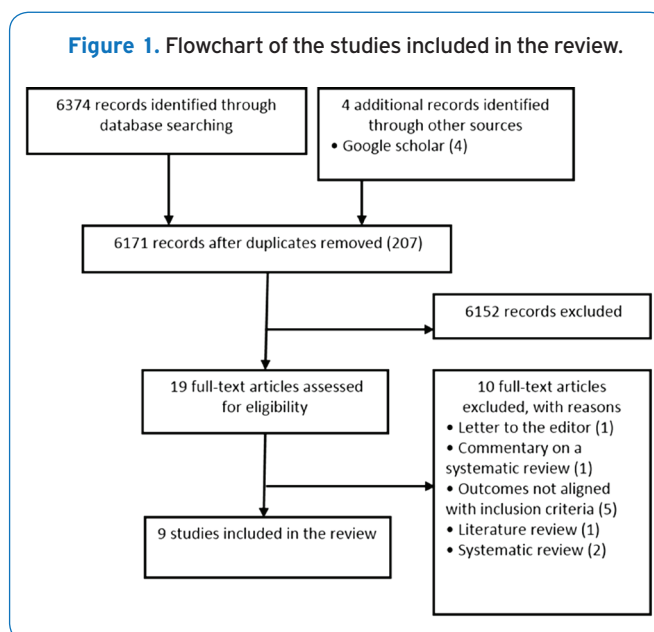


Table 1. Characteristics of included studies.

Author	Study Type	Tendon investigated	Groups /Intervention	Population(s)	Mean (SD)		Outcomes Analysed in Review
					Age in years		
Bryant et al. (47)	Cross-sectional	Achilles	20 Users of monophasic oral contraceptive pill (MOCP) 20 No contraception (non-MOCP)	Pre-menopausal athletic women (runners)	28.0 (4.2) 31.9 (7.3)		Achilles tendon strain Plasma oestrogen levels (pg/ml)
Cook et al. (1)	Cross-sectional	Achilles	16 Users of HRT 37 Non-HRT 8 Users of HRT 24 Non-HRT	Post-menopausal asymptomatic active women (golfers) Post-menopausal asymptomatic inactive women (golfers)	58.0 (8.27) 61.8 (6.36) 58.8 (7.72) 58.9 (6.87)		Achilles tendon diameter (mm) Achilles tendon status: normal/abnormal (% abnormal)
Finni et al. (48)	Cross-sectional	Achilles	14 Users of HRT 14 Non-HRT	Post-menopausal monozygotic female twin pairs (14 pairs)	57.2 (1.8)		Achilles tendon thickness (cm) Achilles tendon CSA (cm <sup>2</sup> ) Serum estradiol (pmol/L) Free estradiol (pmol/L) Estrone (pM)
Hansen et al. (19)	Cross-sectional	Patellar	10 Users of ERT post hysterectomy 10 Non-users of ERT	Post-menopausal women	61 (4) 60 (4)		Patellar tendon force maximum Serum estradiol (nmol/L) Tendon fibril size, density, tendon CSA, tendon stress, tendon strain, tendon stiffness Young's Modulus, Tendon collagen synthesis (FSR, PINP)
Hansen et al. (33)	Cross-sectional	Patellar	15 Users of oral contraceptive pill 15 Non-users of the oral contraceptive pill	Pre-menopausal athletic women (handball players)	23.0 (1.0) 22.0 (1.0)		Patellar tendon CSA (mm <sup>2</sup> ), tendon fibril diameter (nm), tendon stress, strain, stiffness, Young's Modulus Plasma oestrogen (nmol/L), LH, FSH
Holmes & Lin. (49)	Cross-sectional	Achilles	44 Women with Achilles tendinopathy 38 Men with Achilles tendinopathy	Women and Men diagnosed with symptomatic Achilles Tendinopathy	Women = 51.3 Men = 49.5		Prevalence (%) of HRT or oral contraceptive use in people with Achilles tendinopathy
Hansen et al. (35)	Cross-sectional	Patellar	11 Users of oral contraceptive pill 12 Non-users of the oral contraceptive pill	Pre-menopausal young healthy women	24 (2) 24 (4)		Patellar tendon CSA Serum estradiol levels (nmol/L) Tendon collagen synthesis (PINP)
Hansen et al. (20)	Cross-sectional	Patellar	11 Users of oral contraceptive pill 12 Non-users of the oral contraceptive pill	Pre-menopausal young healthy women	24 (2) 24 (4)		Patellar tendon CSA Serum estradiol levels (nmol/L) Tendon collagen synthesis (FSR)
Pingel et al. (34)	Randomised controlled cross over study	Patellar	Oestrogen patch applied to the patellar tendon No oestrogen patch (total of 11 participants in crossover)	Post-menopausal women	65.0 (2.0)		Serum estradiol and (nmol/L) Serum PINP (mgL <sup>-1</sup> ) Whole body tendon collagen synthesis (PINP)

N/A = not applicable, CSA = cross-sectional area, PINP = a measure for amino-terminal propeptide of type I collagen, FSR = Functional synthesis rate, HRT = Hormone Replacement Therapy, ERT = Estrogen Replacement Therapy.

ity was defined as “a variation in the fiber structure of the tendon evident in both the longitudinal and the transverse scans”, commonly seen “as a hypoechoic region or fusiform swelling”<sup>1</sup>. In studies where mean and confidence intervals or standard errors were published, the variance measure was converted to standard deviations to enable comparison of results across the studies<sup>1</sup>. Where studies presented results graphically, data were estimated from the graph<sup>45</sup> and confirmed using GraphClick computer software (Arizona-Software, Version 3.0, 2008), which has been shown to be a valid and reliable tool<sup>46</sup>.

## Results

### Study selection

The number of references considered at each stage of the review and the reason for exclusions are shown in Figure 1. In total, 9 studies were included in the review.

### Study characteristics

Participants across the studies ranged in number from 11 to 85 and had a mean age (SD) between 22.0 (1.0) and 65.0 (2.0) years (Table 1). All studies included in the review compared FSHS (intervention group) with no FSHS (control group). Eight of the nine cross-sectional studies were observational and participants in the intervention groups were taking FSHS prior to undertaking the study. Of these eight, four studies examined the effects of the oral contraceptive pill on tendon in pre-menopausal women<sup>20,33,35,47</sup> and three examined the effects of oral hormone replacement therapy on tendon in post-menopausal women compared to a control group<sup>1,19,48</sup>. The remaining retrospective study investigated the prevalence of oral contraceptive and hormone replacement therapy use in a group of people with Achilles tendinopathy<sup>49</sup>. There was only one study that randomised FSHS and placebo (5 day transdermal oestrogen patch) in a post-menopausal population<sup>34</sup>.

In addition to investigating FSHS, four out of nine studies included results for non-exercise and exercise legs<sup>19,20,34,35</sup>, two reported on active and inactive groups<sup>1,48</sup> and one on the participants' normal jumping leg and contra-lateral leg<sup>33</sup>. Outcomes assessed varied considerably between the studies. The most commonly evaluated outcome was tendon CSA<sup>19,20,34,35,48</sup>.

### Risk of bias assessment

All included studies displayed strong external validity, being representative of pre and post-menopausal populations<sup>1,19,20,33-35,47-49</sup>. Performance bias was low, with 8 out of 9 studies having stated or implied observation of the interventions. A moderate level of detection bias was present among the studies, with only five studies utilising blinded assessors<sup>1,19,33,35,47</sup>. Selection bias varied considerably across the confounding factors incorporated into the analysis. Six out of nine studies indicated the method and dosage of

hormone administration<sup>19,20,33-35,47</sup>. Three papers reported the type of hormone therapy but not the dosage level<sup>1,48,49</sup>. Seven studies recorded the duration of hormone therapy usage prior to participating in the study<sup>1,19,20,33-35,47,48</sup>. All of the studies investigating the oral contraceptive pill, identified the method of data collection and the timing of the menstrual cycle and/or pill cycle<sup>19,20,35,47</sup>. Seven out of nine studies reported baseline activity levels of included participants, which is important for determining the effects that exercise/activity levels have on tendon when combined with a hormone therapy supplement. All studies recorded the weight of included participants but only four studies identified the presence or absence of co-morbidities<sup>20,33,34,49</sup>. Four other studies provided an overarching statement of ‘healthy’ or ‘athletic’ women, assumed to indicate a lack of co-morbidities<sup>1,19,35,47</sup>. Finni et al.<sup>48</sup> reported that their participants had no contraindications to participate in their study. In only one study, were participants randomised to receive the intervention<sup>34</sup>. Five of the eight studies<sup>19,20,33-35</sup> used both legs of the same participant to examine the impact of resistance exercise – having an ‘exercise’ and a ‘non-exercise’ leg, rather than an ‘exercise group’ and a ‘non-exercise’ group (Table 2).

### Tendon outcome analysis

#### Molecular/Histopathological response

Very limited evidence (one low quality study<sup>19</sup>) exists for tendon collagen fractional synthesis rate (FSR), and limited evidence (two low quality studies unable to be combined with meta-analysis<sup>19,34</sup>) for tendon PINP (a measure for amino-terminal propeptide of type I collagen synthesis<sup>50</sup>) in the post-menopausal population. Tendon collagen FSR for the non-exercise and exercise legs was greater in post-menopausal women on FSHS<sup>19</sup>. In pre-menopausal women, tendon FSR was significantly lower for the non-exercise leg for participants not taking the OCP, however no differences were found in the exercise leg. In post-menopausal women, PINP was higher and lower with hormone supplementation<sup>19,34</sup>. Very limited evidence exists for serum PINP in post-menopausal women and tendon fibril density in pre and post-menopausal women, with only one low quality study reporting on each outcome. No differences were found for serum PINP at day 2, 3 or 5 of the transdermal oestrogen patch application (Figure 2)<sup>34</sup>. Although higher in post-menopausal women, no significant differences for tendon fibril density were found in either population group<sup>19</sup>.

#### Mechanical response

Very limited evidence existed for all mechanical response measures (one low quality study per outcome), except tendon strain that had conflicting evidence (two low quality studies, unable to be combined with meta-analysis). Tendon stress was lower and maximum tendon force and Young's modulus significantly lower with FSHS in post-menopausal women<sup>19</sup>. Contrary to this, Hansen et al.<sup>33</sup> found that with FSHS supplementation, tendon stress and Young's Modulus was higher

**Table 2.** Quality assessment and risk of bias for included studies.

Study	External Validity		Internal Validity										
	Representative	Participation rate	Performance		Detection Selection bias/Control of Confounding factors								
			Direct Observation	Blinded Assessors	Method and dosage of FSHS	Duration of HRT or OC usage prior to the study indicated	non-OC users testing procedure timing described	Baseline activity or fitness levels of participants described	Participant co-morbidities, or lack thereof, indicated	Participant weight/BMI	Randomisation of FSHS	Impact of exercise assessed with separate exercise and non-exercise groups	
Bryant et al. (47)	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	×	✓
Cook et al. (1)	✓	×	✓	✓	×	✓	N/A	✓	×	✓	×	×	✓
Finni et al. (48)	✓	×	✓	×	×	✓	N/A	✓	×	✓	×	×	✓
Hansen et al. (19)	✓	✓	✓	✓	✓	✓	N/A	✓	✓	✓	×	×	×
Hansen et al. (33)	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	×	×	×
Holmes & Lin (49)	✓	✓	×	×	×	×	N/A	×	✓	✓	×	×	✓
Hansen et al. (35)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	×	×
Hansen et al. (20)	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	×	×	×
Pingel et al. (34)	✓	✓	✓	×	✓	✓	N/A	×	✓	✓	✓	✓	×

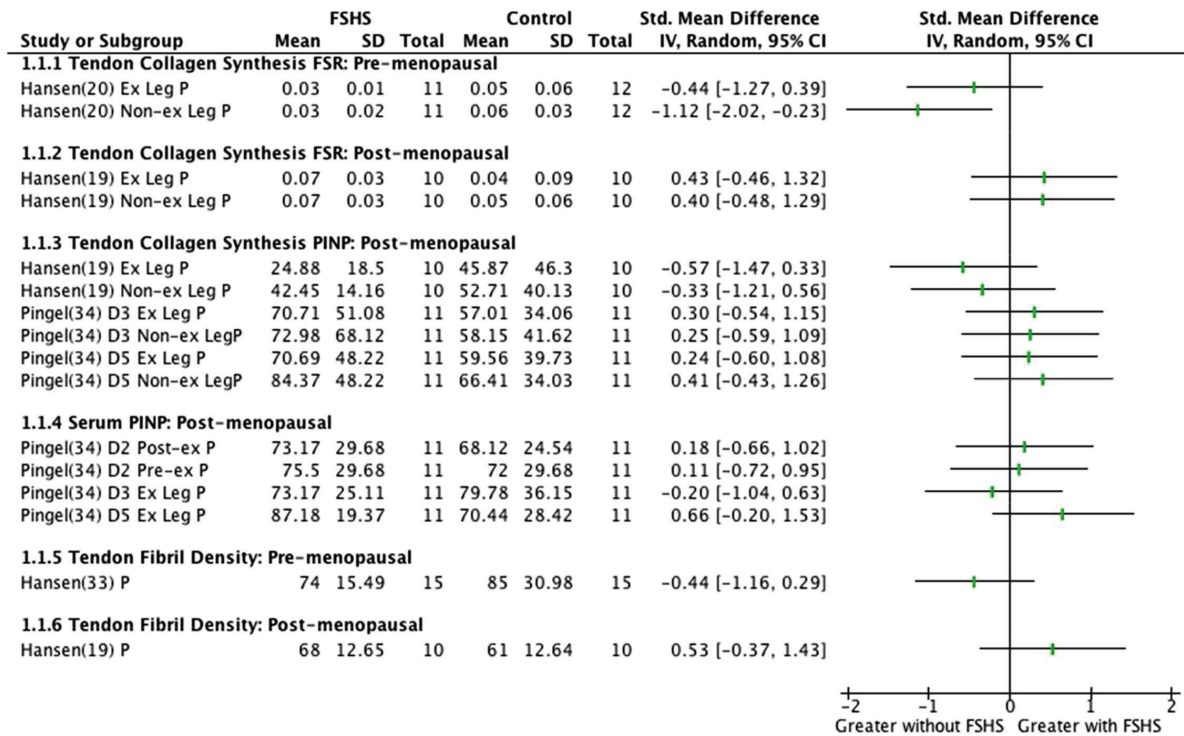
Note: ✓ indicates the measure was adequately addressed in the study. × indicates the measure was not adequately addressed in the study.

Representative: ✓ if study had pre and/or postmenopausal participants but no menopausal participants.

Participation rate: ✓ if 100% of those who were eligible to participate in the study were included in the study.

Direct Observation: ✓ if either be stated or implied.

N/A not applicable to the study.

**Figure 2.** Molecular Response of tendon in pre and post-menopausal women.

Values are means  $\pm$  SD (standard deviation); FSHS = female sex hormone supplementation, Ex = intervention (exercise) leg, Non-ex = non-exercise leg; std. = standard; SD = standard deviation; D = Day; FSR = fractional collagen synthesis; PINP = amino-terminal propeptide of type I collagen; A = Achilles tendon; P = patellar tendon.

in the contralateral leg but lower in the jumping leg in pre-menopausal women, however none of these differences were significant. Differences in pre-menopausal tendon strain was evident with Bryant et al.<sup>47</sup> finding lower strain with FSHS, and Hansen et al. (2013)<sup>33</sup>, found no significant difference. Bryant et al.<sup>47</sup> reported results during two sections of the female hormone cycle – ovulation (where serum oestrogen levels are at their lowest) and menstruation (where serum oestrogen levels are at their highest). No significant differences were identified in tendon strain in post-menopausal women and tendon stiffness in pre and post-menopausal women (Figure 3).

#### Morphological response

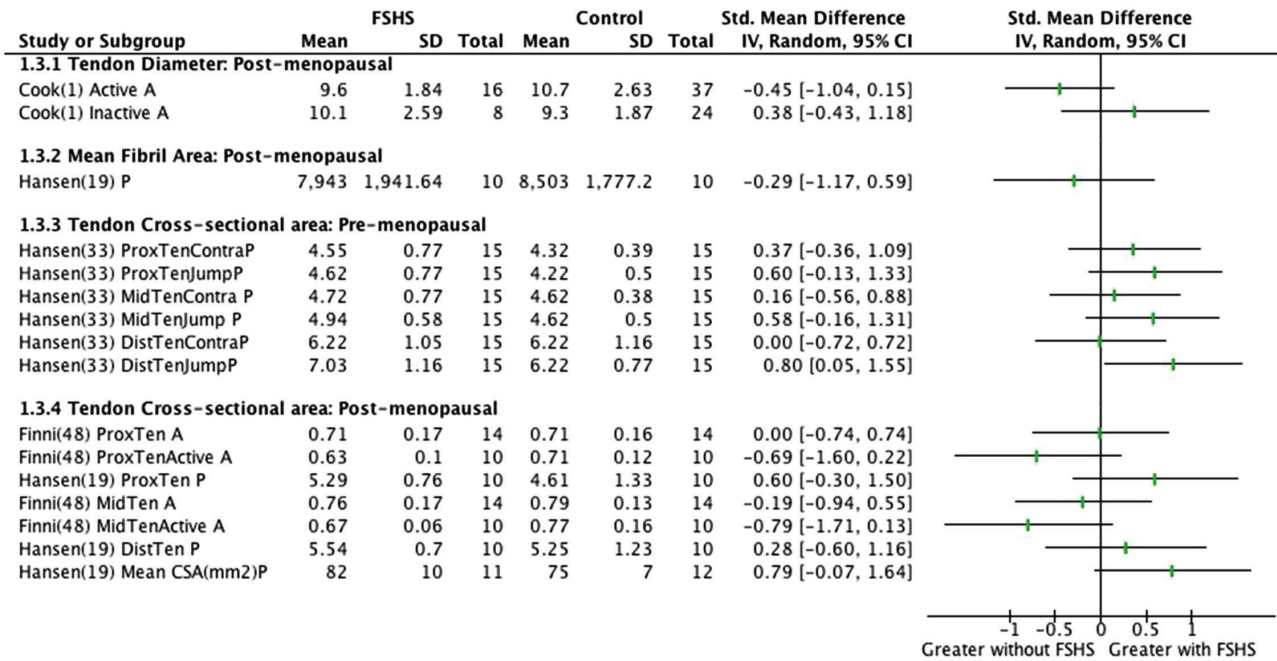
Very limited evidence existed for post-menopausal cross sectional area (middle and distal tendon), tendon diameter and mean fibril size, and pre-menopausal tendon cross sectional area (proximal, middle and distal), with only one low quality study reporting on each of these outcomes. Cook et al.<sup>1</sup> reported that active women on HRT had less tendon abnormality than inactive women on HRT ( $p=0.056$ )<sup>1</sup>. However the odds ratio suggests no significant difference in risk of tendon abnormality (OR=0.31, 95%CI=0.08, 1.08). Inactive participants on hormone replacement therapy were more

likely to have tendon abnormality (OR=5.0, 95%CI=0.91, 27.47) compared to those not on hormone replacement therapy. No significant differences were found in tendon diameter<sup>1</sup> or mean fibril size<sup>19</sup> across both pre and post-menopausal populations.

No significant differences were found for cross-sectional area (CSA) for the non-exercise leg at the proximal, mid and distal parts of the tendon in participants taking FSHS. Cross-sectional area of the proximal tendon of the non-exercise leg in post-menopausal women was the only outcome appropriate for meta-analysis, using a random effects model to account for the heterogeneity between studies<sup>51</sup>. With a sample size of 24<sup>19,48</sup>, there were no significant findings (degrees of freedom:  $df=1.0$ , 95%CI -0.34 to 0.83,  $p=0.41$ ). Conflicting evidence exists for post-menopausal FSHS for the proximal tendon, as indicated by a low level of statistical heterogeneity between the studies,  $I^2=3\%$ <sup>19,48</sup>.

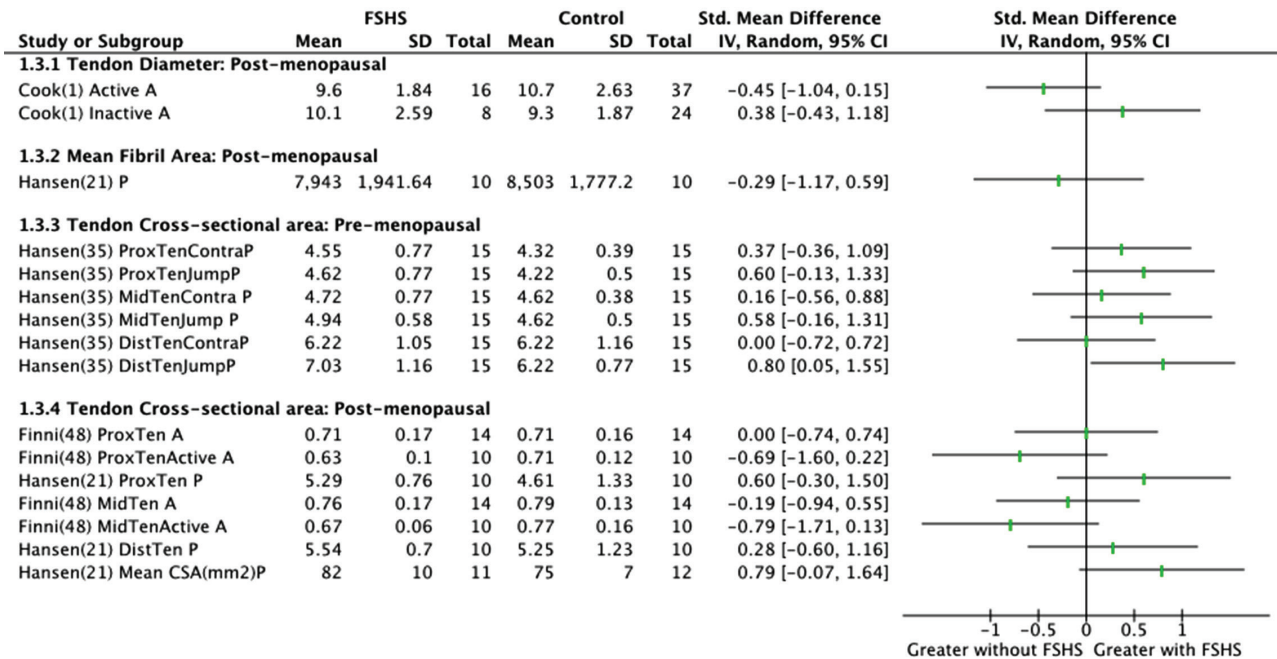
For the exercise leg, pre-menopausal populations taking FSHS (OCP) had significantly higher distal CSA<sup>33</sup>. There was also higher CSA at proximal and mid parts of tendon<sup>33</sup> however differences were not significant. In contrast, CSA was greater, but not significantly so, in a post-menopausal population<sup>48</sup> not taking FSHS (Figure 4).

**Figure 3.** Mechanical Response of tendon in pre and post-menopausal women.



Values are means ± SD (standard deviation); FSHS = female sex hormone supplementation, Jump = normal jumping leg; Contra = contra-lateral leg/non-jumping leg; A = Achilles tendon; P = patellar tendon.

**Figure 4.** Morphological response of tendon in pre and post-menopausal women.



Values are means ± SD (standard deviation); FSHS = female sex hormone supplementation, Prox = Proximal, Mid = Middle, Dist = Distal; CSA = Cross sectional area; ProxTenActive = proximal tendon active; ProxTendon = Proximal tendon; Contra = contra-lateral leg/non-jumping leg; A = Achilles tendon; P = patellar tendon.



Holmes and Lin<sup>49</sup> reported that within their cohort of participants with Achilles tendinopathy, hormone replacement therapy was statistically associated with Achilles tendinopathy in the 60-64 year age group and for the group as a whole (50-74 years).

## Discussion

This review provides a synthesis of the current literature on the effects of FSHS on tendon. Caution should be taken extrapolating these findings, as included studies were mostly observational, underpowered, and had outcomes that were difficult to interpret within the small body of literature available. Similarly, the presence of large confidence intervals in the results, decrease the likelihood of ruling in or out, any physiologically important effects. Using the vanTulder approach to assess level of evidence<sup>39</sup>, this review shows either limited, very limited or conflicting evidence for all outcomes assessed.

Although, the intention was to provide a series of meta-analyses, the range of outcomes, ages and activities across studies, made it difficult to combine data to determine the true effect of FSHS on tendon. Due to this clinical heterogeneity, only one outcome could be evaluated via meta-analysis - CSA of the proximal tendon<sup>19,48</sup>. The results suggest that FSHS has a varied effect on tendon outcomes when compared to no FSHS. Young's modulus and maximum tendon force were lower, and tendon FSR was higher, in post-menopausal women taking FSHS<sup>19</sup>. There was a trend towards tendon PINP being higher with FSHS supplementation<sup>34</sup>, and tendon CSA being higher without FSHS under an exercise condition<sup>48</sup>, in post-menopausal women. Significantly lower FSR for the non-exercise leg was seen in pre-menopausal women<sup>20</sup> and although not significant, there was also a trend for higher tendon CSA with FSHS in this population<sup>33</sup>. Tendon strain results varied between two studies, showing both higher and lower strain in response to FSHS in pre-menopausal women<sup>33,47</sup>. All other findings were not significant.

### *Effects of supplemental oestrogen*

#### Molecular/Histopathological response

Profoundly different FSR of collagen were reported in two studies<sup>19,20</sup>, likely due to differences in sample population: pre-menopausal<sup>20</sup> versus post-menopausal women<sup>19</sup> and type of intervention - OCP (2 mg of oral serum oestradiol) versus ERT (30 µg ethinyl oestradiol or 35 µg ethinyl oestradiol and 0.25 mg norgestimate per day). The risk of bias was moderate in both studies. Neither study randomised the intervention<sup>20,35</sup>, and the use of blinded assessors in Hansen et al.<sup>20</sup> was not explicit.

The outcome measures of tendon collagen synthesis (FSR and PINP) are pragmatic and clinically relevant however, whether they are an accurate measure of what occurs at the tendon tissue level is questionable. Unlike muscle, core tendon is not continuously renewed<sup>52</sup>. It has been reported that intracellular degradation of collagen accounts for up to 10-

40% of newly synthesised tendon collagen<sup>53-55</sup>. Therefore measures of protein synthesis rates using stable isotopes, as reported in two of the included papers<sup>19,20</sup>, may overestimate collagen synthesis as proteins may be degraded prior to being incorporated into the extracellular matrix<sup>52</sup>. Additionally, serum measures (s-PINP) may provide a more systemic reflection of collagen synthesis that occurs in many tissues. Similarly, in a study on bone turnover, Bennell et al.<sup>56</sup> found no association between measures of serum collagen cross-links (pyridinoline: Pyr and deoxypyridinoline: D-Pyr) in urine samples and the development of stress fractures. However, as these are measures of bone remodelling throughout the skeleton, local changes at the site of the stress fracture are possible, similar to what may occur in tendon. As a consequence, results from included studies should be reviewed with caution as these outcome measures (tendon collagen FSR and PINP, and serum PINP), may not be a true reflection of collagen synthesis in the tendon tissue.

#### Mechanical response

Biomechanical properties of tendon were investigated in three out of eight included studies<sup>19,33,47</sup>. Although widely researched in the literature, there is no consensus on the optimal level of tendon stiffness, stress and strain, or a method to accurately measure such properties. However, these properties are highly participant specific; may be responsive to participant age, injury history, training loads, and immobilisation<sup>57</sup>. This review found that the biomechanical properties of Young's modulus and maximum tendon force were significantly reduced in the presence of oestrogen in post-menopausal women<sup>19</sup>. For pre-menopausal women, results for tendon stress, stiffness and Young's modulus were inconclusive<sup>33</sup>. Bryant et al.<sup>47</sup> reported significantly higher Achilles tendon strain in the control group compared to the OCP user group ( $p=0.035$ ), however in our analysis, findings were only significant during menstruation. Articles investigating the mechanical properties of Achilles tendon highlight the large variation in methods used to quantify tendon strain and stress<sup>58-61</sup>. Thus, it is difficult to evaluate whether changes in these properties are a barrier or facilitator to tendon health.

#### Morphological response

Tendon diameter, mean fibril size, tendon abnormality and CSA were evaluated in this review. Neither tendon diameter nor mean fibril size was affected by FSHS. Tendon CSA was the most evaluated outcome in this review. There is debate as to whether higher or lower tendon CSA is a helpful morphological adaptation or a marker of pathology. Previous studies have shown larger tendon CSA with heavy resistance training compared to light resistance training<sup>62</sup> and remain unchanged with prolonged endurance training<sup>63</sup>. Challenging this interpretation, is the study by Heinemeyer et al.<sup>52</sup> that reported adult core tendon collagen renewal is extremely limited and the formation of tendon collagen occurs within the first 17 years of life<sup>52</sup>.

### Prevalence of tendinopathy

Holmes and Lin<sup>49</sup> found most of the participants in their study diagnosed with Achilles tendinopathy used FSHS. As this was a retrospective study it is unknown if those taking FSHS had other comorbidities that may have an additional impact on their Achilles tendon health. The risk of bias analysis revealed a presence of selection bias, as the baseline activity/fitness level of the included participants and the method, dosage and the duration hormone supplementation use, was unknown.

### Effects of rest and exercise conditions on tendon

Several studies reported on the additional impacts of no-exercise and exercise on tendon (secondary aim) in those people taking FSHS. Different methods were used to investigate this: five studies presented results for each leg of a participant (a non-exercise/rest leg and an exercise leg)<sup>19,20,33-35</sup>; two studies divided the participants into a control (inactive) group and active group or sub-group of people<sup>1,48</sup> and one study described their participants as active, however only reported on the primary outcome of this review. Only one significant result was found with the addition of exercise - pre-menopausal populations taking the OCP had significantly higher CSA. Thus, evidence to suggest that exercise impacted considerably on tendon morphology was not heavily substantiated.

Issues can arise when reporting on both legs of a participant, particularly if the exercise applied to one leg has systemic and/or cortical effects, resulting in changes to the contralateral leg<sup>64,65</sup>. This has been evident in animal studies where tenocyte hypercellularity and vascular proliferation have been measured in response to exercise in one limb in rabbits<sup>66</sup>. Significantly larger tenocyte numbers were reported after three and six weeks of training in both the exercised and unexercised limb. Also, an increase in vascularity and VEGF-mRNA of the tendon tissue was found. These findings were indicative of bilateral tendinosis like changes in both the exercised and non-exercised leg, suggesting it would be inappropriate to use the non-exercised limb as a control. In addition, it is believed that unilateral exercise may activate neural circuits causing plastic changes in the primary motor cortex<sup>64,65</sup> and altering the excitability of cortical<sup>67-72</sup> and spinal<sup>67,73-75</sup> motor pathways to the contralateral limb. Thus, in this review, where studies have implemented unilateral strength training in combination with supplemental oestrogen<sup>19,20,34,35</sup>, the effects on the non-exercised limb may be exaggerated, and accurate interpretation of the effect of exercise with supplemental oestrogen on tendon, more difficult.

### Limitations

Of the outcomes assessed in this review, many were only evaluated by 1-2 studies, meaning that a definitive effect of FSHS on each outcome could not be determined. In addition, there is a possibility of publication bias (i.e. unpublished stud-

ies with insignificant findings or small effect sizes).

It is difficult to interpret whether changes in the outcome measures described in this review represent a positive or negative change. For exercise in isolation, contradictory findings have been observed for mechanical properties such as stiffness and Young's Modulus<sup>76</sup>. These contradictory findings highlight the difficulty of dichotomising response to FSHS as positive and negative.

### Summary

This review provides no strong evidence to suggest a positive or negative link between FSHS and tendon outcomes. The overall findings of this review must be interpreted in light of methodological limitations of included studies, study heterogeneity, and the moderate risk of bias. Studies reported on tendon response outcomes relating to oral contraceptive pill supplementation<sup>20,33,35,47</sup>, oral hormone replacement therapy<sup>1,35,48</sup> and a transdermal oestrogen patch<sup>34</sup>. Although dosage and type of hormone administered may have been defined, no attempt to standardise the dosage of oral contraceptive across study participants was made. In the randomised controlled crossover study, it was reported that a recommended dose of oestrogen replacement therapy of 50 µg/24hour patch was administered to all participants<sup>34</sup>. However, the source of such recommendation and the optimal dosage and duration of FSHS administration to affect molecular, mechanical and morphological tendon outcomes, positively or negatively are unknown. Therefore it is difficult to determine the true effect of FSHS on tendon. Only one of the nine studies randomised the hormone therapy intervention<sup>34</sup>. Risk of bias was higher in the remaining eight studies as they recruited participants who were already taking FSHS prior to the commencement of the study. As a consequence, the intervention and control groups are likely to vary on known and unknown confounding factors at baseline. The decision to take, or reasons for taking, FSHS (medical, contraceptive and life-style) potentially divides participants prior to inclusion in the studies.

### Conclusions

The effect of oestrogen supplementation on tendon is contradictory and inconsistent. Few studies reported the effects of oestrogen on the molecular, mechanical and morphological responses of tendon. Most mechanical and molecular tendon response outcomes were not significantly different between FSHS and no supplementation. Although, results suggest some benefit of FSHS for higher tendon CSA in pre-menopausal women and reducing tendon abnormality in post-menopausal women, results must be viewed with caution. Included studies were mostly observational, underpowered and had outcomes that were difficult to interpret within the small body of literature available. This review suggests there is a need for further studies to understand the effects of FSHS on tendon tissue at a mechanical, morphological and molecular level.

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