

# Knee health and associations with female-specific health, physical, psychological and social-gendered factors in women runners: the TRAIL-W cohort study protocol

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## ABSTRACT

**Introduction** Running is a popular recreational activity worldwide, with women's participation growing rapidly over the past decade. Compared with men, women runners are more likely to sustain a running-related injury, such as bone stress and knee injuries. Following a serious knee injury and subsequent surgery, women also experience worse knee and health-related outcomes than men. However, little is known about the intersection of female-specific health, physical, psychological and social-gendered factors with knee health in women runners with and without a history of knee surgery.

**Methods and analysis** Building on the established 'TRAjectory of knee health in runners' (*TRAIL*) prospective cohort study and designed with patient and content-expert partners, the nested *TRAIL-W* study will comprehensively explore the associations of multiple factors (ie, female-specific health, physical, psychological and social gendered) with knee health (symptoms and structural features), device-measured running load and running-related pain in women runners. Where appropriate, we will explore sex and/or gender differences. Alongside their scheduled *TRAIL* 6-monthly data collection, all active *TRAIL* female and male participants will be invited to complete a once-off '*TRAIL-W* survey' and attend an additional laboratory-based assessment. The survey will include questionnaires measuring psychological, social gendered and, for women only, female-specific health factors. The laboratory assessment will measure body composition, bone mineral density and blood biomarkers. A subset of women will be invited to participate in qualitative interviews to understand women runners' experiences of female-specific health factors and their association with running behaviour.

**Ethics and dissemination** Findings from *TRAIL-W*, approved by the La Trobe University Human Ethics Committee, will address critical research gaps by describing and exploring the diverse factors that may influence women runners' knee health.

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Running has physical and psychological benefits, including a 25%–40% reduced risk of premature mortality; reduced depression, anxiety and stress, but is associated with a high risk of running-related injury and pain. Compared with men, women runners are more likely to sustain a running-related injury, such as bone stress and knee injuries, and have slower injury recovery times.

## WHAT THIS STUDY ADDS

⇒ Building on the established TRAjectory of knee health in runners (*TRAIL*) cohort, the nested *TRAIL-W* study will be the first cohort study to explore the associations among female-specific health, physical, psychological and social-gendered factors, with the trajectory of knee health (symptoms and structural features), running load and running-related pain over time in women runners, including exploring sex and gender differences where appropriate.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Findings from *TRAIL-W* will address a critical research gap by comprehensively describing and exploring the diverse factors that may influence women runners' knee health. The codesign of the study with runners, patient/public lived-experience partners and clinical and research experts reflects real-world priorities for sports and exercise medicine research.

## BACKGROUND

Running is a globally popular recreational activity, with women's participation in major events growing by up to 58% over the past decade.<sup>1</sup> For people with a serious

knee injury (eg, anterior cruciate ligament (ACL) or meniscal tear), running may be favoured over returning to pivoting sports.<sup>2</sup> Running has physical and psychological benefits, including a 25%–40% reduced risk of premature mortality; and reduced depression, anxiety and stress,<sup>3</sup> but is associated with a high risk of running-related injury and pain.<sup>4</sup> Compared with men, women runners are more likely to sustain a running-related injury, such as bone stress and knee injuries, and have slower injury recovery times.<sup>4,5</sup> Following a serious knee injury and surgery, women also experience worse symptoms, including poorer knee-related quality of life and function up to 5-years following surgery.<sup>6</sup>

The prognosis for knee health, when considered in terms of knee *symptoms* and joint *structural features*, is unknown in women runners, especially after knee surgery. Our 2023 consensus recommendations on the prevention of osteoarthritis (OA) and promotion of knee health following traumatic knee injury<sup>7</sup> could not identify any gender- or sex-specific prognostic factors for worse *symptoms* or joint *structural* outcomes. It is possible that female-specific health factors, such as gynaecological/menstrual, obstetric, breast and pelvic floor health, could directly or indirectly influence knee-joint health (symptoms and structural features), running-related pain and/or device-measured running loads (eg, distance, intensity and frequency of runs) for women runners. For example, menstrual disturbances and hormonal fluctuations may influence pain perception<sup>8</sup> and bone health<sup>9</sup>; knee pain is experienced by ~25% of pregnant women runners<sup>10</sup>; a well-fitting bra can improve running biomechanics and performance<sup>11</sup> and urinary incontinence/fear of leakage can influence running loads (eg, self-limiting intensity and frequency) and biomechanics (eg, altered kinematics and stride length).<sup>12,13</sup> Other factors that disproportionately affect women runners, such as low-energy availability and/or relative energy deficiency in sport,<sup>14</sup> may directly or indirectly influence knee health via physical (eg, changes in strength, biomechanics/movement pattern and bone mineral density (BMD))<sup>15</sup> and/or psychological factors (eg, fear of movement and disordered eating).<sup>16,17</sup> For example, disordered eating is linked to menstrual health disturbances and bone stress injuries, with potential long-term consequences, such as osteoporosis.<sup>9</sup>

Women runners' health and self-reported running behaviour, including opportunities and timing of runs, are further challenged by pervasive social-gendered factors, such as safety concerns and/or stereotypical-gendered roles.<sup>18,19</sup> For example, following childbirth, women have greater childcare responsibilities and less time to commence or return to regular running, possibly resulting in muscle weakness and knee pain.<sup>20–22</sup> Women may also face unique barriers rarely considered during rehabilitation and recovery from knee injury or surgery,

including competing life demands/caring responsibilities, sex and/or gender-specific strength training needs and the influence of menstrual and/or obstetric health.<sup>21,23</sup>

To address these research gaps, we are expanding our longitudinal prospective cohort running study—TRAjectory of knee heaLth in runners (*TRAIL*)—to include a nested cohort study: *TRAIL-W*. *TRAIL* was established to compare knee health in runners with (surgery group) and without (control group) a history of knee surgery at baseline and at 4- and 10-year follow-up. Knee health outcomes include knee *symptoms*, as measured by the Knee Injury and Osteoarthritis Outcome Score (KOOS), and knee-joint *structural features*, as measured by magnetic resonance imaging (MRI). *TRAIL* was intentionally designed to include equal numbers of women and men.<sup>24,25</sup> *TRAIL-W* will collect and explore data across female-specific health, physical, psychological and social-gendered factors (figure 1) in interested *TRAIL* participants (as appropriate) and includes primary, secondary and tertiary objectives. For *TRAIL-W*, the *TRAIL* surgery and control groups will be combined, with surgery status a modifying variable. Refer to Box 1 for how we use sex and gender terms throughout this article.

## Objectives

### Primary (women-specific) objectives

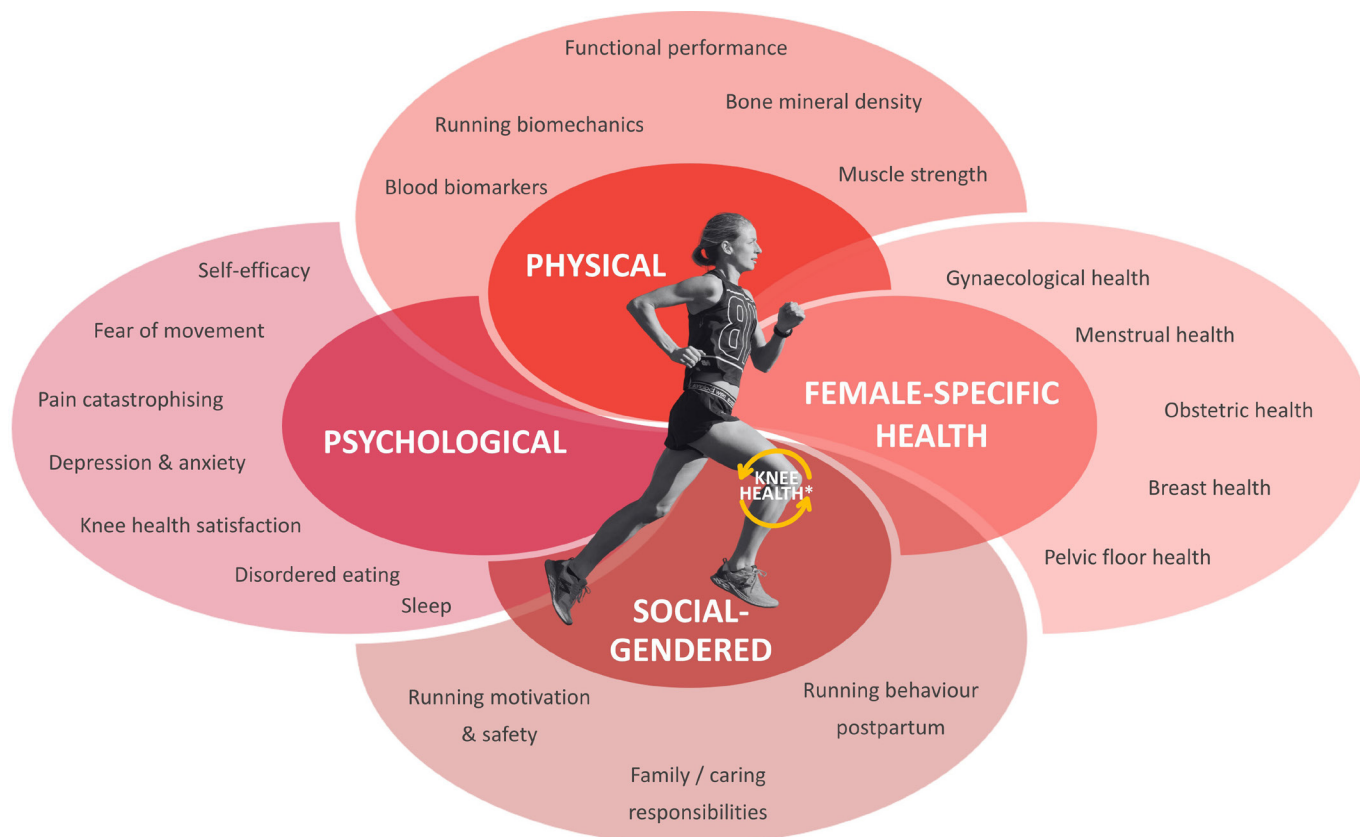
In women runners, to:

1. describe the prevalence of female-specific health factors, including gynaecological, menstrual, obstetric, breast and pelvic floor health;
2. explore associations of female-specific health, physical, psychological, social-gendered factors and self-reported running behaviour (eg, opportunities and timing) with knee health (ie, symptoms and structural features), knee health over time, device-measured running loads (ie, distance, intensity and frequency) and running-related pain;
3. explore associations between female-specific health and social-gendered factors with psychological factors (eg, anxiety, depression, kinesiphobia, self-efficacy, disordered eating, sleep quality and quantity and pain catastrophising) and how they might interact to influence knee health;
4. explore the experiences of female-specific health factors and the association with running behaviour via qualitative interviews.

### Secondary (sex and/or gender comparison) objectives

In all runners, to:

1. describe sex and/or gender differences in social-gendered factors (eg, running safety and caring roles) and self-reported running behaviour (eg, opportunities and timing);
2. explore sex and/or gender differences in the strength or direction of associations between key factors (social gendered, psychological, physical factors and relevant



**Figure 1** Visualisation of factors in the TRAIL-W study that may contribute to knee health (\*includes symptoms and structural features) in women runners with and without a history of knee surgery.

### Box 1 Terminology based on sex and gender equity in research guidelines.<sup>61</sup>

#### Sex

- ⇒ Biological attributes (eg, hormones, anatomy and reproductive anatomy).
- ⇒ Terms include female, male and intersex.
- ⇒ Collected at TRAjectory of knee heALth in runners (*TRAIL*) enrolment time point.

#### Gender

- ⇒ Socially constructed roles, behaviours and norms.
- ⇒ Terms include woman, man and non-binary.
- ⇒ Gender may not align with a person's sex assigned at birth.
- ⇒ To be collected at *TRAIL-W* time point.

#### In this study

- ⇒ We recognise that sex and gender are distinct but interrelated concepts and binary categories may not capture the diversity of human experience. To acknowledge the intersection of both constructs, we will use the terms *woman/women* and *man/men* throughout the article.
- ⇒ The terms *female* and/or *male* will be used when referring to biological sex-related factors (eg, female-specific health factors, such as menstrual, pelvic and obstetric health).

pelvic health factors, eg, urinary incontinence) and self-reported running behaviour.

#### Tertiary (low-energy availability) objectives

In all runners, to describe features of low-energy availability and associations with:

- ▶ participant general health characteristics (eg, injury history and comorbidities);
- ▶ knee health (ie, symptoms and structural features) and running-related pain;
- ▶ physical factors (eg, biomechanics, muscle strength, functional performance and bone mineral density (BMD)), including device-measured running loads (ie, distance, intensity and frequency), female-specific health and psychological factors;
- ▶ social-gendered factors, including self-reported running behaviour (eg, opportunities and timing).

#### Patient and public involvement

The design of the *TRAIL-W* study was informed by a consumer advisory group (CAG) and a scientific advisory group (SAG). The CAG and SAG will contribute to future data analysis, interpretation, coauthorship, dissemination and knowledge translation.

### Consumer advisory group

The Women's Knee Health CAG includes six cisgender females (age range 29–40 years; five with ACL rupture and surgery lived experience and one physiotherapist with experience treating women post-ACL injury). CAG members have a range of experiences with running, knee injuries, surgery and rehabilitation.<sup>21</sup> The CAG's priorities for research on women's knee health informed the current project, including understanding the role, influence and impact of: (1) physical activity and load; (2) female-specific health factors (eg, menstrual/gynaecological/pregnancy); (3) social-gendered factors; (4) nutrition and body image and (5) pain on women's short- and long-term knee health.<sup>21</sup>

### Scientific advisory group

The TRAIL-W SAG comprises academic and clinical experts (MHay, DM, JD, JST, AC, AG, RB, MC, LT, SW and RS) in female-specific health factors, including gynaecological, obstetric, breast and pelvic floor health. The experts recommended using valid questionnaires and data collection methods when available and contributed to the design of bespoke survey items based on their specific areas of expertise.

## METHODS

### Study design

TRAIL-W is a nested cohort within the established longitudinal TRAIL cohort study. Our published TRAIL study protocol describes in detail all existing methods, outcomes and data collection time points.<sup>24</sup> Key TRAIL cohort characteristics at enrolment and baseline have also been published.<sup>25</sup> The TRAIL-W project is approved by the La Trobe University Human Ethics Committee (HEC-19524). The PROgnosis REsearch Strategy (1 and 2) framework guided the TRAIL-W protocol development.<sup>26 27</sup>

### Participants

Participants who completed baseline testing for TRAIL<sup>24 25</sup> (n=209, figure 2) will be invited to complete additional TRAIL-W measures. We will aim for gender balance and anticipate that at least 100 TRAIL participants will enrol in TRAIL-W.<sup>24 25</sup> Participants will provide written informed consent.

### Data collection, outcomes and exposures

Tables 1,2 summarise all existing outcomes/exposures and their respective time points collected as a part of TRAIL and proposed new measures and data collection time points for TRAIL-W. Enrolled TRAIL-W participants will complete a once-off 'TRAIL-W survey' (See online supplemental file A), alongside their scheduled TRAIL 6-monthly data collection time point (tables 1,2). Participants will also be invited to attend La Trobe University for additional laboratory-based data collection, including the assessments of body composition, BMD and blood

biomarkers. A subset of women will be invited to participate in qualitative interviews.

### EXISTING MEASURES FROM THE TRAIL STUDY

TRAIL-W will use the following measures from TRAIL (tables 1,2). Refer to the published TRAIL protocol<sup>24</sup> for full details.

#### Measures of knee health (all participants; table 1).

Knee-related symptoms are collected as a primary outcome of TRAIL via the KOOS at TRAIL enrolment, baseline, 6-monthly between baseline and 4-year follow-up time points and yearly from 4-year until the 10-year follow-up time point. The KOOS has five subscales, including pain, symptoms, activities of daily living, sport and recreation function and knee-related quality of life, with scores ranging from 0 (worst) to 100 (best).<sup>28</sup> An additional subscale—KOOS Patellofemoral<sup>29</sup>—will measure patellofemoral-specific outcomes.

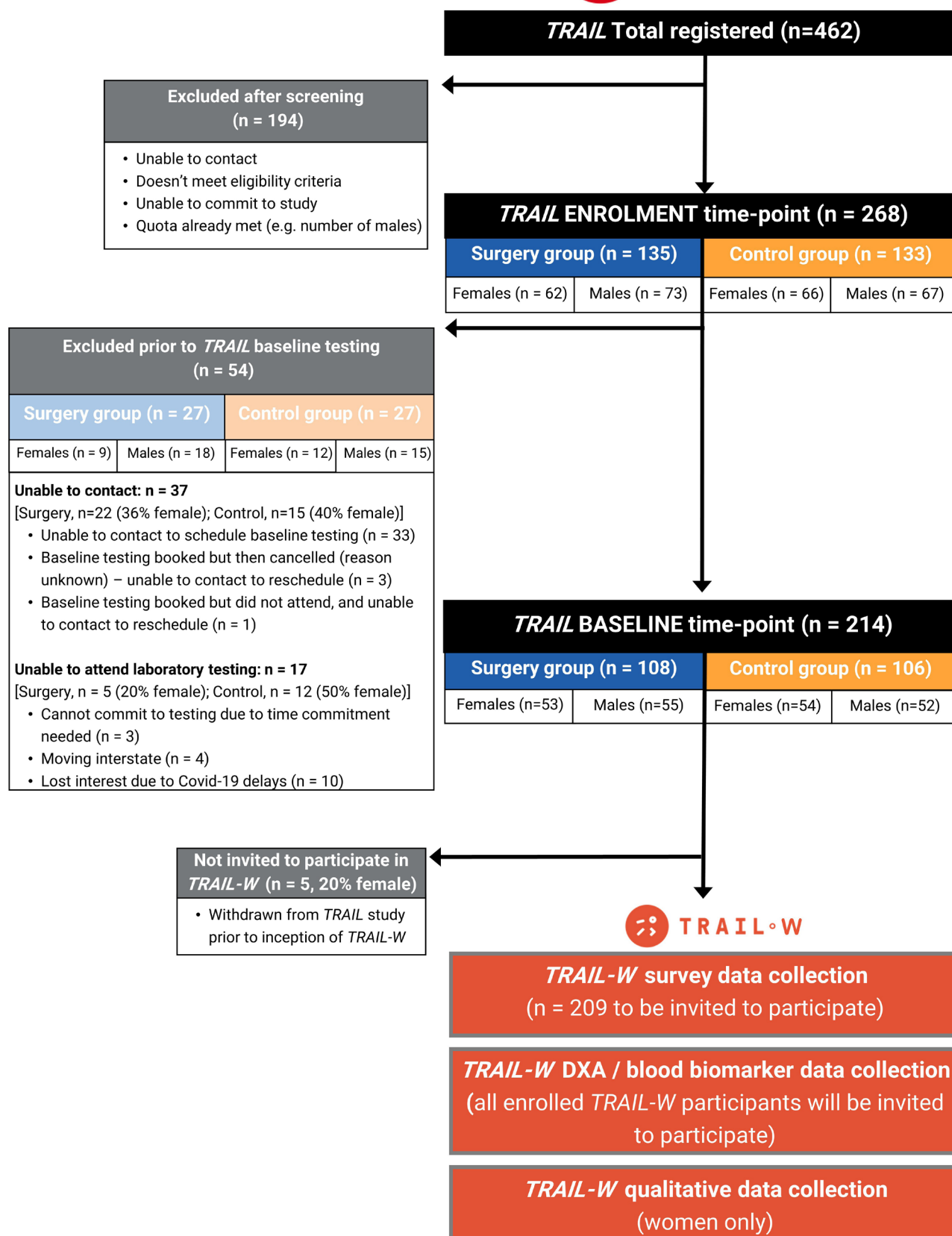
Knee-joint structural features are collected and analysed as a TRAIL primary outcome at baseline, 4-year and 10-year time points. These include: (1) cartilage collagen content and orientation, defined by quantitative changes in T2 MRI relaxation times; (2) knee cartilage thickness and bone shape and (3) knee morphological features associated with OA (eg, cartilage defects, bone marrow lesions and osteophytes), scored with the MRI OA knee score (MOAKS).<sup>30</sup> For the primary TRAIL-W objective, we will use the MRI time point closest to the completion of TRAIL-W data collection.

#### Other exposures and/or outcome measures (all participants; tables 1,2).

Device-measured running load will be extracted daily from TRAIL enrolment until the 10-year follow-up via participants' wearable smart watch syncing to an app (Teamworks AMS, Teamworks Innovations Australia Pty Ltd.), which provides external load (ie, distance (km); duration (min); pace (average min/km) and cadence (average steps/min)) and internal load (ie, average and maximum heart rate).

Running-related pain/symptoms and their impact on participation (self-reported) are collected monthly from TRAIL enrolment through the 4-year follow-up, adapted from the Oslo Sports Trauma Research Centre Overuse Injury Questionnaire (questions 1 and 4 only).<sup>31</sup> Participants record the extent of running-related pain and running participation difficulties due to such pain that they have experienced in the last 7 days for three body areas (knee, Achilles and other). Each item uses a four-point scale with weighted values, scored from 0 (no problems) to 25 (severe problems/unable to participate). If participants report pain, they indicate its location on a bespoke body pain map (knee/Achilles) or a body chart (other).

Physical factors are collected at the TRAIL baseline time point, including 3-D overground running



**Figure 2** TRAIL prospective cohort study and TRAIL-W participant flowchart adapted from De Oliveira Silva *et al*'s study.<sup>25</sup> DXA; dual-energy X-ray absorptiometry; TRAIL, TRAJjectory of knee heaLth in runners.



**Table 1** TRAIL and TRAIL-W outcomes and exposures, including data collection time points

Description	Measure	Frequency of data collection					TRAIL		
		Once	Monthly	Once	Monthly	Once	Trail-W	TRAIL 4-year follow-up	TRAIL 10-year follow-up
<b>Participant demographics/characteristics</b>									
Height and body mass	Self-reported and/or laboratory measured	x		x					
Sex	Bespoke questionnaire		x						
Gender	Bespoke questionnaire		x						
Demographics (eg, race/ethnicity)	Bespoke questionnaire	x		x					
Sexual orientation, living arrangements	Bespoke questionnaire			x					
Running behaviour history (eg, years running)	Bespoke questionnaire	x						x	x
Running footwear, accessories and training surfaces	Bespoke questionnaire	x						x	x
Other training (eg, strength)	Bespoke questionnaire	x						x	x
<b>General health-related characteristics</b>									
Knee injury and surgery history	Bespoke questionnaire; SMDCS and the OSIIICS <sup>62</sup>	x						x	x
Bone stress injury history	Bespoke questionnaire; SMDCS and OSIIICS <sup>62</sup>	x						x	x
Other lower limb injury history	Bespoke questionnaire; SMDCS and OSIIICS <sup>62</sup>	x						x	x
History of OA	Bespoke questionnaire: family history; history of structural OA diagnosis	x						x	x
Knee symptoms	Self-reported: pain, swelling, crepitus	x						x	x
General health and comorbidities	FCI <sup>46</sup>			x					
Generalised joint hypermobility	Hypermobility 5PQ <sup>47</sup>			x					
Activity and sporting levels	Tegner activity scale <sup>48</sup>			x					
Smoking status and alcohol intake	Bespoke questionnaire			x					
<b>Primary trail outcomes</b>									
<b>Primary TRAIL knee health outcomes</b>									
Knee-related symptoms, including pain, function and disability	KOOS <sup>28</sup>	x		x				x	x
Knee-joint structural features; MRI—cartilage thickness and quality	MOAKS (semiquantitative scoring), <sup>30</sup> T2 MRI mapping.			x				x	x
<b>Exposure or secondary outcomes (depending on the research question)</b>									
<b>Primary TRAIL exposure</b>									

Continued

**Table 1** Continued

	TRAIL enrolment	TRAIL baseline	Trail-W	TRAIL 4-year follow-up	TRAIL 10-year follow-up
	Frequency of data collection				
	collected daily†				
Device-measured running loads, including external running load (eg, distance, intensity and frequency) and internal running load (eg, heart rate)					
Self-reported running-related pain/symptoms and impact on participation					
Running-related pain and participation (knee, Achilles and other)	x	x	x	x	x
Training load data via wearable smart device—GPS <sup>63</sup>					
Adapted from the OSTRC overuse injury questionnaire <sup>31</sup> and bespoke pain maps for knee, Achilles and whole body if pain is indicated.					
<b>Qualitative data collection</b>					
Semistructured 1:1 interview		x*			
Beliefs and experiences related to running/OA <sup>64</sup> Experiences regarding pelvic health symptoms impacts on running behaviour.					
x indicates the data collected on all participants. F indicates the data collected on female participants only. †Indicates exposure data collected daily from the enrolment time point until the 10-year time point. ‡Indicates data collection as a once-off event completed between the TRAIL baseline and 4-year follow-up testing. Refer to De Oliveira Silva et al <sup>24</sup> for full details on TRAIL outcomes and exposure. †Indicates exposure data collected daily from the enrolment time point until the 10-year time point. FCI, Functional Comorbidity Index; GPS, Global Positioning System; KOOS, Knee Injury and Osteoarthritis Outcome Score; MOAKS, MRI osteoarthritis knee score; OA, osteoarthritis; OSIICS, Orchard Sports Injury and Illness Classification System; OSTRC, Oslo Sports Trauma Research Centre; 5PQ, 5-part Questionnaire; SMDCS, Sport Medicine Diagnostic Coding System; TRAIL, TRAjectory of knee health in runners.					



**Table 2** TRAIL and TRAIL-W outcomes and exposures, including data collection time points

Description Measure	TRAIL enrolment		TRAIL baseline		Trail-w	TRAIL 4-year follow-up		TRAIL 10-year follow-up
	Once	Monthly	Once	Monthly	Once	Monthly	6 monthly	Once
Frequency of data collection								
<b>Description Measure</b>								
Exposure or secondary outcomes (depending on the research question)								
<b>Female-specific health factors</b>								
Menstrual health					F			
Menopause/perimenopause					F			
Low-energy availability					F			
Obstetric health/pregnancy (past and current)					F†			
Breast pain (symptoms and severity) during running-related activity					F			
Knowledge of sports bra design and bra fit					F			
Pelvic health history (including incontinence, polycystic ovary syndrome and endometriosis)					F†			
Pelvic health symptoms and impact on running participation, performance and confidence					F†			
<b>Physical factors</b>								
Biomechanics during running (easy and fast paced)								x
Muscle strength (quadricep/hamstring peak isometric torque/rate of torque development)								x
Functional performance (vertical hop, side hop and hop for distance tests)								x
Clinical knee measures (knee flexion and extension range of motion; knee-joint line tenderness on palpation. One-leg rise test. Crepitus.)								x
BMD								x

Continued

**Table 2** Continued

Description Measure	Frequency of data collection			
	TRAIL enrollment	TRAIL baseline	Trail-w	TRAIL 10-year follow-up
Blood biomarkers		x*		
Systematic inflammation	Inflammatory markers -cytokines (IL-1 $\beta$ , IL-6, IL-8, IL-10 and TNF- $\alpha$ ),			
	hsCRP		x	
Iron deficiency	Iron studies (FBE, iron, transferrin and ferritin)		x	
Thyroid function	TSH, FT4 and FT3		x	
Bone Health	CTx: P1NP and serum 25-hydroxyvitamin D		x	
Metabolic function	Fasting serum glucose		x	
<b>Psychological and well-being factors</b>				
Fear of movement and reinjury	TSK <sup>33</sup>	x	x	x
Self-efficacy	KSES <sup>32</sup>	x	x	x
Pain catastrophising	PCS <sup>45</sup>		x	
Anxiety	GAD-7 <sup>43</sup>		x	
Depression	PHQ-9 <sup>44</sup>		x	
Beliefs about OA and running	Bespoke questionnaire adapted from Esculier <i>et al</i> <sup>66</sup>	x		x
Disordered eating	EDE-Q <sup>42</sup>		x	
Knee acceptability symptom state	PASS <sup>35</sup>	x	x	x
Sleep quantity, quality and disturbance	ASSQ <sup>34</sup>	x	x	x
<b>Social-gendered factors</b>				
Self-reported running behaviour: opportunities/timing of runs, influence of childbirth and running alignment with desire	Bespoke questionnaire		x	
Safety concerns, toilet considerations	Bespoke questionnaire		x	
Family and caregiving responsibilities	Bespoke questionnaire		x	

x indicates the data collected on all participants.

F indicates the data collected on female participants only.

\*Indicates data collection as a once-off event completed between the TRAIL baseline and 4-year follow-up testing. Refer to De Oliveira Silva *et al*<sup>24</sup> for full details on TRAIL outcomes and exposures.

†Indicates that male participants are asked applicable questions, for example, urinary incontinence or if their partner had given birth.

APFG, Australian Pelvic Floor Questionnaire; ASSQ, Athletic Sleep Screening Questionnaire; BMD, bone mineral density; CTx, C-terminal telopeptide of type I collagen; DXA, dual-energy X-ray absorptiometry; EDE-Q, Eating Disorder Examination Questionnaire; GAD-7, Generalised Anxiety Disorder Assessment-7; hsCRP, high-sensitivity C reactive protein; ICIQ, International Consultation on Incontinence Questionnaire; KSES, Knee Self-Efficacy Scale; LEAF-Q, Low-Energy Availability in Females Questionnaire; OA, osteoarthritis; PASS, Patient Acceptable Symptom State; PCS, Pain Catastrophising Scale; PFBQ, Pelvic Floor Bother Questionnaire; PHQ-9, Patient Health Questionnaire-9; P1NP, procollagen type 1 N-terminal propeptide; TRAIL, TRAJECTORY of knee health in runners; TSK, Tampa Scale for Kinesiophobia.

biomechanics (kinematics and kinetics), lower limb functional performance (vertical, forward and side hopping; and one-leg rise), isometric knee extensor and flexor muscle strength (Biodex System 4 Pro, New York, NY, USA) and blood biomarkers (cytokines: IL-1 $\beta$ , IL-6, IL-8, IL-10 and TNF- $\alpha$ ).

Psychological (and well-being) factors, including self-efficacy, fear of movement/reinjury, sleep (including quality, quantity and disturbance) and knee health satisfaction, are collected via valid questionnaires, respectively, including the knee self-efficacy scale,<sup>32</sup> Tampa scale for kinesiophobia,<sup>33</sup> athlete sleep screening questionnaire<sup>34</sup> and the patient acceptable symptom state<sup>35</sup> at *TRAIL* enrolment, baseline, 6-monthly between baseline and 4-year follow-up time points and yearly from 4-year until the 10-year follow-up time point.

### NEW DATA COLLECTION FROM THE *TRAIL-W* STUDY

*TRAIL-W* SURVEY (see tables 1 and 2, online supplemental file A)—using valid questionnaires, unless indicated

#### Female-specific health factors (including menstrual, pelvic, obstetric and breast health)

The low-energy availability in females questionnaire (female participants only) is a 25-item screening tool designed to identify female athletes at risk of problematic low-energy availability. It consists of three domains: injury history, gastrointestinal function and menstrual function. Scores range from 0 to 25, with higher scores indicating greater risk. A total score of  $\geq 8$  indicates the individual is at risk for problematic low-energy availability/relative energy deficiency in sports.<sup>36</sup>

The International Consultation on Incontinence Questionnaire—Urinary Incontinence Short Form (ICIQ-UI SF) (all participants) is a four-item self-report measure designed to assess the subtype, frequency, severity and impact of urinary incontinence in daily life over the prior 4 weeks.<sup>37</sup> Items 1–3 are scored based on leakage frequency (0–5 scale), leakage volume (0–6 scale) and overall impact on quality of life (0–10 scale), resulting in a total score ranging from 0 to 21. Higher scores indicate more severe urinary incontinence and greater impact on quality of life. The fourth item is not scored but identifies leakage subtype (ie, stress, urgency and mixed). The ICIQ-UI SF is used in clinical and research settings to assess urinary incontinence severity, monitor treatment outcomes and screen individuals at risk.<sup>37</sup> An additional question, ‘Do you ever experience urine leakage (incontinence)?’ was added to establish the prevalence of urinary incontinence.

The pelvic floor bother questionnaire (female participants only) is a self-report measure used to assess the presence and extent of bother in the daily life of pelvic floor symptoms (eg, urinary, bowel and prolapse

symptoms).<sup>38</sup> If participants answer ‘yes’ to each described symptom, they rate their level of bother on a scale of 0 (not at all) to 5 (a lot). No valid tool to investigate pelvic pain in athletes currently exists; therefore, a question from the urogenital distress inventory<sup>39</sup> was used to establish the location and functional source of pain (ie, bladder, bowel, uterus or vagina). Additional follow-up questions adapted from Dakic *et al*<sup>40</sup> for participants experiencing symptoms included: (1) symptom presence during running, playing sport or exercising (yes or no); (2) the degree of symptom impact on participation/performance during running, sport or exercise (0: not at all–5: a lot) and (3) how the symptom impacts running, sport or exercise participation/performance (eg, loss of concentration, distraction, avoidance, reduced intensity and changes in exercise habits).

Two items from the Australian pelvic floor questionnaire (all participants) will be adapted to assess additional pelvic health symptoms, including the frequency of bladder infections (item 12) and frequency of bowel straining (item 18).<sup>41</sup> Individualised frequency scales score items from 0 to 3, with higher scores indicating greater symptom frequency.

Obstetric health (female participants only) will be explored via bespoke questions after responding to ‘have you given birth’, including type of birth (vaginal and caesarean), perinatal obstetric factors, such as labour/delivery interventions (eg, forceps and episiotomy), and outcomes (third or fourth degree perineal tear).

Breast pain (cyclic mastalgia, exercise induced and bra-related chafing), frequency and severity, bra-wearing behaviour and knowledge and ranking of the current sports bra for support/comfort will be collected via an expert-informed, bespoke questionnaire (female participants only). Breast pain frequency and severity will be ranked using a Likert scale of 0 (never) to 5 (all the time) and a numeric rating scale of 0 (no pain) to 10 (worst pain possible). Participants’ knowledge will be assessed using a series of statements with response options of ‘agree’, ‘disagree’ or ‘neither agree nor disagree’.

#### Psychological factors (all participants)

Eating disorder examination questionnaire Short is a 12-item self-report measuring eating disorder symptoms over the previous 7 days. Scores range from 0 to 36, with higher scores indicating greater severity of eating disorder symptoms (eg, restraint, body dissatisfaction and weight concern).<sup>42</sup>

Generalised anxiety disorder assessment is a seven-item scale measuring generalised anxiety over the previous 2 weeks. Scores range from 0 to 21, with higher scores indicating greater severity of anxiety symptoms (eg, worry, nervousness and tension).<sup>43</sup>

Patient health questionnaire is a nine-item scale that measures depressive symptoms over the previous 2 weeks. Scores range from 0 to 27, with higher scores indicating greater severity of depressive symptoms (eg, sadness, anhedonia and fatigue).<sup>44</sup>

Pain catastrophising scale is a 13-item scale measuring exaggerated negative thoughts and feelings about pain. Scores range from 0 to 57, with higher scores indicating greater levels of pain catastrophising (eg, rumination, magnification and helplessness about pain).<sup>45</sup>

### Social-gendered factors (all participants)

Motivation, safety and other sociocontextual factors associated with pregnancy, postpartum, family/caring responsibilities and living arrangements that may influence participants' running behaviour, including timing and opportunities to run, will be explored via bespoke questionnaires to all participants where relevant. For example, participants who indicate that their partner has given birth will also be asked postpartum running behaviour questions, for example, 'Has having a child/children affected your running behaviour? For example, if, when, where, how and how much?'

### General health-related characteristics (all participants)

The functional comorbidity index (FCI) is an 18-item checklist to assess the presence of comorbid health conditions that may affect physical functioning. Each item represents a specific chronic condition (eg, diabetes) and is scored as 0 (absent) or 1 (present). Total scores range from 0 to 18, with higher scores indicating a greater burden of comorbidity associated with poorer physical function. The FCI was developed for use with musculoskeletal and rehabilitation populations in clinical and research settings.<sup>46</sup>

Generalised joint hypermobility will be assessed using the valid five-part questionnaire, which consists of five yes/no items (one point per yes answer). A score of  $\geq 2$  indicates the presence of generalised joint hypermobility.<sup>47</sup>

Tegner activity scale is a single-item questionnaire used to assess an individual's level of physical activity.<sup>48</sup> It consists of an 11-point ordinal scale ranging from 0 (disability due to knee problems) to 10 (competitive sports at a professional or elite level). Participants will be asked to complete the Tegner for their current level of activity and their retrospective level of activity at the time of *TRAIL* enrolment.

General health/other variables will be collected, including gender identity, self-reported height and body mass, smoking status, alcohol intake and a history of lower limb musculoskeletal injury and surgery since enrolment in *TRAIL*.

### TRAIL-W LABORATORY DATA COLLECTION—ADDITIONAL PHYSICAL FACTORS (ALL PARTICIPANTS)

*TRAIL-W* participants will be invited to attend La Trobe University (Melbourne, Australia) to undergo dual-energy X-ray absorptiometry scans and blood biomarker collection. Further eligibility to participate in laboratory testing includes providing written informed consent and satisfying a safety screen to rule out contraindications for additional testing (eg, pregnancy/breastfeeding and

annual limit for ionising radiation exposure—online supplemental file B). Participants will be advised to: (1) fast overnight before testing, (2) hydrate and (3) not perform any strenuous physical activity or physical activity lasting  $>30$  min within 24 hours leading up to testing.

### Body composition and BMD

DXA scans will assess whole-body composition (fat mass, including body fat (%) and absolute fat mass (kg), lean soft tissue mass and BMD) and BMD at the lumbar spine and hip (total hip and femoral neck). Standardised protocols for body composition<sup>49</sup> and BMD<sup>50</sup> will be applied. DXA scans will be performed by a trained researcher (JPH) who holds a Victorian Government radiation licence using a Hologic Horizon W QDR 4500A instrument (Hologic Horizon, Hologic Inc., Bedford, MA, USA). The machine uses extremely small doses ( $<1\%$  of the yearly dose) of radiation, with the total effective dose calculated by a Medical Physicist. Scans will comply with relevant codes of practice and best practice guidelines.<sup>51 52</sup>

### Blood biomarkers

Blood-serum biomarkers of systemic inflammation will be collected, including high-sensitivity C reactive protein. Standard markers of iron status (iron, transferrin and ferritin), thyroid function (TSH, FT4 and FT3), bone health (C-terminal telopeptide of type I collagen, Procollagen type 1 N-terminal propeptide and total serum 25-hydroxyvitamin D concentration) and metabolic function (fasting serum glucose) will be collected. A phlebotomy-trained medical doctor (IC) will collect a 30-mL sample via a venepuncture from an appropriate arm vein (eg, antecubital or cephalic). Venous blood samples will be kept at room temperature for 30 min to allow clotting, then centrifuged at 3000 rpm for 10 min to separate the serum supernatant. Serum will be pipetted into 2-mL Eppendorf tubes and stored at  $-80$  °C at La Trobe University (Melbourne, Australia) for later analysis.

### QUALITATIVE DATA COLLECTION (SUBSET OF WOMEN PARTICIPANTS; TABLE 1).

Semistructured one-on-one interviews, guided by a prestructured interview guide (developed by the research team, CAG and SAG members), will be conducted to understand women runners' experiences with female-specific health factors (eg, pelvic health and pregnancy; see online supplemental file C) and their association with their running behaviour. Purposive sampling will be used to recruit women runners with varying female-specific health experiences identified from the *TRAIL-W* survey results. Interviews will be conducted on Zoom by an experienced qualitative researcher, audio recorded and transcribed verbatim.

### Data analysis plan

For descriptive objectives, descriptive statistics and visualisations will summarise participant characteristics, outcome

scores and prevalences. Exposures of interest will be related to repeated measures of *TRAIL* knee health outcomes (including symptoms and structural outcomes, device-measured running loads and running-related pain) using general or generalised hierarchical (time points nested within individuals) models with the appropriate distribution and linked function. Latent trajectory analysis may also be used, as appropriate, to relate exposures to the subgroups of participants with different patterns of change in outcomes.<sup>53</sup> Dynamic structural equation modelling may be used in the case of intensive longitudinal data (ie, running load and running-related pain).<sup>54</sup> Objectives related to sex-and/or gender-based differences in outcomes will involve cross-sectional (outcomes measured once) or hierarchical (repeated measures) regression models using sex/gender as an independent variable and evaluating sex/gender interactions with other exposure variables. For analyses involving cross-sectional data, general or generalised linear models with the appropriate distribution and linked function will be used. An aetiological framework<sup>55</sup> will be adopted for investigations of exposures of interest and will seek to minimise bias by adjustment for potential confounding as appropriate for each exposure examined. An estimation approach to statistical inference will be used, with 95% CIs provided for all statistical estimates of association.<sup>56</sup> For qualitative analyses, reflexive thematic analysis using Braun and Clarke's six-step approach<sup>57</sup> will be used to develop and refine themes, supported by NVivo.

## DISCUSSION

Despite the growing participation of women runners worldwide, research that considers female-specific health and social-gendered factors in the context of knee health and running-related pain is scarce. Building on the established *TRAIL* cohort, the nested *TRAIL-W* study will be the first prospective cohort study to comprehensively explore the associations of multiple female-specific health factors, physical, psychological and social-gendered factors, with knee health and running-related load and pain in women runners. Leveraging a key strength of the *TRAIL* study, which was intentionally designed to redress historical sex/gender imbalance in sports and exercise medicine research by including equal numbers of women and men runners,<sup>24 25</sup> *TRAIL-W* builds on this commitment by collecting additional data across multiple female-specific health factors. While previous studies have examined isolated factors, such as pregnancy-related pain<sup>58</sup> or urinary incontinence in runners,<sup>12</sup> no prior research has combined multiple female-specific health factors, particularly in the context of knee health.

Additional laboratory data, including blood biomarkers and BMD measures, allow the comprehensive exploration of associations with other prospectively collected outcomes, including device-measured running loads (smartwatch), knee-joint structural features (MRI) and symptoms (KOOS). For example, prior studies are retrospective, have a small sample size (n=10)<sup>59</sup> or are mostly male (84%).<sup>60</sup> *TRAIL-W*'s unique combination of objective data, in a sample of ≥100

runners, allows the exploration of multiple factors associated with the knee health of runners.

Our study has limitations that should be acknowledged. Embedding a nested cohort study within an existing longitudinal study may overburden participants and thereby increase the risk of follow-up loss for *TRAIL* primary outcomes. The codesign approach with the CAG/SAG helps mitigate this by including only meaningful and appropriate measures and by optimising data collection timing—invites for *TRAIL-W* will be sent concurrently with the already scheduled *TRAIL* 6-monthly data collection time point to minimise unwanted participant contact. We acknowledge that some exposures may change over time (eg, menstrual health); therefore, limiting exploration of causation—*TRAIL-W* data collection is a single time point, whereas running load, knee symptoms and structural outcomes are longitudinal. We acknowledge potential limitations in sample size for subgroup analyses. *TRAIL*'s sample size of >200 was based on detecting knee symptoms and structural change at 4 years. While we aim to maximise participation in *TRAIL-W*, the anticipated sample size of ~100–150 participants may limit the precision of statistical estimates for some objectives. While we will use valid measures where possible, many female-specific health questionnaires rely on self-report and may be affected by recall bias, particularly historical exposures, such as the age of menarche (first period) or the time of returning to running after childbirth. Bespoke questionnaires have been developed when no valid measure exists or when measurement properties are unknown; however, expert input from the SAG and pilot testing aim to optimise content validity.

*TRAIL-W* is informed by and codesigned with lived-experience consumers (CAG)<sup>21</sup> and content experts (SAG) to enhance the relevance and validity of the additional data collection. This approach strengthens the study's content validity by ensuring that selected measures reflect meaningful outcomes and priorities for women runners with lived experience of knee surgery, which may also facilitate the recruitment and retention of participants.<sup>21</sup>

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