TITLE:

ACL injury risk in elite female youth soccer: changes in neuromuscular control of the knee following soccer specific fatigue

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ABSTRACT
Fatigue is known to influence dynamic knee joint stability from a neuromuscular perspective and electromechanical delay (EMD) plays an important role as the feedback activation mechanism that stabilizes the joint. The aim of this study was to investigate the influence of soccer specific fatigue on EMD in U13, U15 and U17 year-old female soccer players. 36 youth soccer players performed eccentric actions of the hamstrings in a prone position at 60, 120 and 180°/s before and after a soccer specific fatigue trial. Surface electromyography was used to determine EMD from the semitendinosus, biceps femoris and gastrocnemious. A time x age x muscle x velocity RMANOVA was used to explore the influence of fatigue on EMD. A significant main effect for time ($p = 0.001$) indicated that EMD was significantly longer post compared with pre fatigue [58.4% increase]. A significant time x group interaction effect ($p = 0.046$) indicated EMD was significantly longer in the U13 age group compared with the U15 ($p = 0.011$) and U17 ($p = 0.021$) groups and greater post fatigue. Soccer specific fatigue compromised neuromuscular feedback mechanisms and the age-related effects may represent a more compliant muscle-tendon system in younger compared with older girls, increasing risk of injury.
INTRODUCTION
Anterior cruciate ligament (ACL) injury is a common and potentially traumatic sports related injury, presenting with substantial short- and long-term morbidities (Griffin et al. 2005). ACL tears tend to occur during activities including sudden acceleration and deceleration, rapid changes of direction, jumping and landing tasks; where rapid and unanticipated movement responses of the medial and lateral hamstring muscles are necessary to stabilize the knee joint and successfully counteract the extreme load forces generated (McLean et al. 2010; Smith et al. 2012). During these movements numerous muscle actions occur with differing co-contraction strategies required to stabilise the joint.

It has therefore been postulated that the hamstrings reaction time is one of the most important primary risk factor associated with ACL tears (Hughes and Watkins, 2006). Specifically, longer hamstrings reaction times may negatively influence the muscle’s ability to quickly stabilize the knee against the large external loads generated during sporting tasks and subsequently might increase the risk of tear (McLean et al. 2010; Besier et al. 2003, Blackburn et al. 2004). Reflective muscular responses, sometimes referred to as pre-activation, occur as quickly as 20ms after initiation of the stimulus and before load is exerted (Shultz and Perrin, 1999). Feedback or reactive motor control strategies alter muscle activation in response to a situation that loads the lower extremity joints such as the knee (Hewett et al. 2005). Electromechanical delay (EMD) is defined as the time between the onset of muscle activity and the onset of force generation by that muscles action (Zohu et al. 1995), and can vary from 30ms to a few hundred ms (Shultz and Perrin, 1999). Considering this additional time lapse and the need to develop sufficient muscular tension rapidly enough to provide dynamic joint stability, EMD should be considered when evaluating muscular responses to an imposed perturbation or injurious stress (Shultz and Perrin, 1999).

A number of adult studies have demonstrated a significantly longer EMD in women than men, albeit during isometric or concentric muscle actions (Zohu et al. 1995; Blackburn et al. 2009; Bell and
Jacobs, 1986; Granata et al. 2002; Winter and Brookes, 1991). However, among the aforementioned studies, only Blackburn et al. (2009) reported data for the hamstrings during isometric muscle actions and they showed that EMD did not differ significantly between sex. Given the important eccentric role that the hamstrings play in stabilizing the knee it is important to examine the EMD of the hamstring during eccentric muscle actions (Mesfar and Shirazi-Adl, 2006).

Considering the rapid rise in ACL reconstruction cases during the teenage years (approximately 50 reconstruction cases at 12y compared to 550 cases at 17y) and the 2-8 times greater incidence per hours of athletic exposure in females compared to males (Renstrom et al. 2008), it is somewhat surprising that few studies have examined the age related changes in EMD in females. A number of studies have reported significant age related effects in EMD with longer EMD evident in younger boys compared with men (Zohu et al. 1995; Falk et al. 2009). There appears to be only one study that has reported differences in EMD between prepubertal girls and adult females (48% change), albeit for the plantarflexors (Waugh et al. 2013). The longer EMD in children has been attributed to excitation-contraction coupling and muscle fibre conduction velocity (Cohen et al. 2010). However, no studies have examined changes in EMD in female youth following a fatigue related task.

It has been well recognised that injury is most paramount in the final stages of sports performance which coincides with when muscle fatigue is present (Small et al. 2010). As muscles contribute to joint stability, neuromuscular fatigue is often suggested as a risk factor for non-contact ACL injuries (Alentorn-Geli et al. 2009; Yu et al. 2002). The few adult studies that are available have all indicated that fatigue significantly increases EMD (Howatson, 2010; Zohu et al. 1996) and this has been attributed to a number of mechanisms including failure of muscle action potentials (Horita and Ishiko, 1987) and/or impaired excitation-contraction coupling (Kent-Braun, 1999). To date no studies have examined the effects of fatigue on EMD in children, and specifically not in elite female youth soccer players, who might be classified as ‘at risk’, due to the high number of incidence per hours of
exposure (Myer et al. 2013). Therefore, the aim of this study was to explore the effects of soccer specific fatigue on EMD of the hamstrings during eccentric muscle actions in elite youth female soccer players.

MATERIALS AND METHODS

Participants

Thirty-six females aged 12-17 years from an English Football Association Centre of Excellence were recruited to participate in this study. Players were recruited from three age groups; U13’s \( n = 14 \), U15’s \( n = 9 \) and U17’s \( n = 13 \). Verbal consent was obtained from the club prior to approaching players, followed by written parental consent and player assent. Ethical approval was obtained from the institutions Research Ethics Committee. All participants completed a health questionnaire prior to testing. There were two exclusion criteria in this study: (1) histories of orthopedic problems, such as episodes of hamstrings injuries, fractures, surgery or pain in the spine or hamstring muscles over the past six months; and (2) self reported presence of delayed onset muscle soreness (DOMS) at a testing session. Participants were instructed to avoid their regular training regimens throughout the experimental period and not to take part in any vigorous physical activity 48 hours proceeding each testing day. Age was computed from date of birth and date of testing. Stature and body mass were measured according to the procedures of Weiner and Lourie (1981) using a Stadiometer (Holtain Harpenden, Crymych, UK) and calibrated balance beam scales (Weylux Birmingham, UK). Sitting height was measured with a sitting height table (Holtain Harpenden, Crymych, UK). Age from peak height velocity (PHV) was predicted using the equation of Mirwald et al. (2002).

Study Design

Participants were required to visit the laboratory on two separate occasions; once for a habituation session and then once for the pre/post fatigue testing. The purpose of the habituation session was to familiarise the participants with the testing protocol on the isokinetic dynamometer to reduce the effect of learning on the test data as well as introduce them to the SAFT\(^{90}\). On the test days participants
performed baseline EMD tests and isokinetic/EMG cycles in a pre-fatigued state. They then performed the SAFT\textsuperscript{90} followed by post-fatigue isokinetic/EMG testing.

**Procedures**

The assessment of EMD of the dominant limb was performed using a Biodex System-3 isokinetic dynamometer (Biodex Corp., Shirley, NY, USA) and a wireless 8-channel Delsys electromyography telemetry system (Delsys Myomonitor III, Delsys Inc., Boston, MA, USA). The dynamometer and EMG data were interfaced by feeding the analogue data directly from the dynamometer into the Universal Input Unit via a trigger box (Delsys, Boston, MA). This method ensures that the data from the EMG and dynamometer were completely time aligned making it possible to determine the onset of EMG activity in relation to the onset of torque production. Before and after the testing procedure commenced, the dynamometer and the EMG device were calibrated according to their respective manufacturer's instructions.

Participants were secured in a prone position on the dynamometer with the hip passively flexed at 10-20\(^\circ\). The prone position (10-20\(^\circ\) hip flexion) was selected instead of a seated position (80-110\(^\circ\) hip flexion) as: (a) the prone position is more representative of the hip position during running/sprinting in contrast with a seated position; and (b) a prone position replicates the knee flexor and extensor muscle length-tension relationships which occurs in the late phase and the early contact phase of sprinting, and when landing or pivoting, which is when the ACL experiences its greatest rate of loading (Worrel et al. 1990, 1989).

The axis of rotation of the dynamometer lever arm was aligned with the lateral epicondyle of the knee. The force pad was placed approximately 3 cm superior to the medial malleolus with the foot in a relaxed position. Adjustable strapping across the pelvis, posterior thigh proximal to the knee and foot localised the action of the musculature involved. The range of movement was set from 90\(^\circ\) knee flexion (initial position) to 0\(^\circ\) (0\(^\circ\) was determined as maximal voluntary knee extension for each
participant). All settings were noted during the practice session so that they were identical throughout experimental trials.

Surface EMG was obtained from the semitendinosus, biceps femoris and gastrocnemious using bipolar and preamplified electrodes with a fixed interelectrode spacing of 10 mm (DE-02, Delsys, Bagnoli-8, Boston, MA). The electrodes were attached parallel to the muscle fibers and over the dorsomedial muscle bulge at two thirds of the proximodistal thigh length for the semitendinosus, and at the dorsolateral side of the thigh at one half of the proximodistal thigh length for the biceps femoris (Merletti and Parker, 2004). The visually largest area of muscle belly was selected using an isometric action against a fixed lever arm. The ground electrode was placed on the lateral malleolus of the ankle. Each electrode placement was marked with permanent ink during the familiarisation session and re-marked at the end of each testing session to ensure consistent placement on subsequent testing days. Electrodes and cables were secured with surgical tape to avoid movement artifacts.

Before the assessment of EMD all participants performed a “zero offset” function to establish a zero baseline from each of the EMG channels during 10 s of stationary lying. The EMG and dynamometer data were acquired at a sampling rate of 1000 Hz. The dynamometer data were lowpass filtered at 10 Hz (4th order, zero phase lag, Butterworth), and the root-mean-square amplitude for each muscle activity was calculated as follows: the raw EMG signals were measured in a band of 20 to 450 Hz, full-wave rectified, high-pass filtered (4th order, zero phase lag, Butterworth) to remove movement artefacts with a cut-off frequency of 20 Hz, and smoothed with a 100-millisecond RMS algorithm.

After this baseline calculation process, participants were instructed to resist as hard and quickly as possible the knee extension movement generated by the arm of the dynamometer by eccentric action of the hamstrings throughout the full range of motion immediately after receipt of a visual (trigger box) signal. The visual signal, was given randomly within 1-4 s of the ‘ready’ command, and defined
the beginning of data acquisition. Participants were instructed to relax and not exert force on the level arm prior to the visual signals in order to avoid pre-activation of the muscle. Visual inspection of the EMG signal was used to be confident that there was minimal EMG activity prior to movement of the lever arm. If the investigators could observe that pre-activation was taking place, they would remind the participant to relax before starting the lever arm.

Three maximal voluntary eccentric knee flexion muscle actions were performed at 60°/s, 120°/s and 180°/s with 10 s rest between each action and 30 s rest between velocities. After each eccentric muscle action, the tested limb was passively returned to the initial position. The EMD was defined as the time interval between the onset of EMG activity (increase of 15µV above the baseline value) and torque development (time taken [milliseconds] to generate 9.6 Nm torque) (Zohu et al, 1997). The mean of the 2 trials with the closest EMD values for each participant were subsequently used as reliability studies have reported better consistency of a measure when the mean value from several trials (two or more) rather than the single highest or lowest value is used (Sole et al. 2007). Reliability for EMD measures have ranged from 3.1-6.5% depending on muscle action and movement velocity (Howatson et al, 2009).

**SAFT^90 Protocol**

The SAFT^90 is based on time-motion analysis data obtained from English Championship level match play via Prozone and has been validated to replicate the fatigue response of soccer match-play (Small et al. 2010). The design of the course is based around a shuttle run over a 20m distance, with the incorporation of four positioned poles for the participants to navigate using movements such as walking, jogging, side stepping and sprinting (Figure 1).

***Figure 1 Here***

The course was performed with the participant performing either backwards running or sidestepping around the first field pole, followed by forward running through the course, navigating the middle
three field poles. The movement intensity and activity performed by the participants whilst completing the SAFT\textsuperscript{90} course were maintained using verbal signals on an audio CD. The audio CD contains a 15 min activity protocol which was repeated randomly and intermittently in order to last for the duration of a game the participant usually competes in, including a passive rest interval equivalent to those experienced on a match day (Table 1). The coach and a member of the research team provided strong verbal encouragement throughout the protocol to help maintain participant effort. Participants completed the course in groups of two or three staggered at 30 minute intervals. All participants were videoed during the SAFT\textsuperscript{90} to determine group mean values for total distance covered.

***TABLE 1 HERE***

**Statistical Analysis**

The distributions of raw data sets were checked using the Kolomogorov-Smirnov test and demonstrated that all data had a normal distribution (p > 0.05). Descriptive statistics including means and standard deviations were calculated for each measure. A 3 x 3 x 3 x 2 (muscle; age; velocity and time) repeated measures analysis of variance (RMANOVA) was used to explore interaction and main effects for EMD. Significant interaction or main effects were further examined using Bonferroni-corrected post hoc tests. Main effects were calculated irrespective of time, angle and velocity. Percent change scores are also reported. The level of significance for all statistical testing was set at alpha level p ≤ 0.05.

**RESULTS**

Participant characteristics can be seen in table 2 and indicates a significant difference between groups for all outcome variables.

***TABLE 2 HERE***
Fatigue was defined as a reduction in torque production and this was evident in all participants irrespective of age and movement velocity (total % decrease in concentric and eccentric torque was 17% and 26% respectively). Mean (SD) data for EMD by age group, pre and post fatigue can be found in Table 3. Data is presented for each muscle group and divided by movement velocity. RMANOVA revealed a significant time x group interaction effect ($F_{2,34} = 3.404, p = 0.046$). No other interaction effects were observed. Post hoc analysis revealed that EMD was significantly longer in the U13 age group compared with the U15 ($p = 0.011$) and U17 ($p = 0.021$) groups and this difference was greater post fatigue (percentage increase in EMD from pre to post fatigue was 66% [U13], 43% [U15] and 61% [U17] respectively). These data can be seen in Figure 2.

***Figure 2 Here***

A significant main effect for time ($F_{1,35} = 10.031, p = 0.001$) and group ($F_{2,34} = 6.356, p = 0.005$) were also observed. Post hoc analysis revealed irrespective of group, muscle or movement velocity EMD was significantly longer post fatigue compared with pre fatigue ($p = 0.001$ [58.4% increase]). Likewise, irrespective of time, muscle or movement velocity EMD was significantly longer in the U13 age group compared with the U15 ($p = 0.011, 158 ± 66ms versus 113 ± 39ms [15.8% longer]$) and U17 ($p = 0.021, 158 ± 66ms versus 120 ± 40ms [24.1% longer]$) age groups. There were no significant differences in EMD between the U15 and U17 age groups ($113 ± 39ms versus 120 ± 40ms [Δ = 6.2%]$). No significant ($p > 0.05$) main effects for muscle ($132±51ms$ [BF], $133±52ms$ [ST], $127±63ms$ [G]) or movement velocity ($139±69ms$ [60°/s], $122±46ms$ [120°/s], $131±51ms$ [180°/s]) were found.
DISCUSSION

The current study indicates that soccer specific fatigue significantly increases the EMD post fatigue, compromising neuromuscular control required to stabilise the joint, in female youth soccer players. These effects were significantly greater in the youngest (U13) age group compared with the older (U15 and U17) age groups. A significant main effect for time was found, indicating that the EMD was longer following the fatigue task [Δ = 58%], irrespective of age, muscle or movement velocity. It is suggested that this lengthening of EMD post fatigue, which is frequently reported in the adult literature (Howatson, 2010; Zohu et al. 1996), is due to metabolic inhibition of the contractile process and excitation-contraction coupling failure (Kent-Braun, 1999). This detrimental effect of fatigue and change in neuromuscular performance may represent an increased risk of injury. For example, research has identified that well-timed activation of the hamstring muscles can protect the ACL from mechanical strain by stabilising the tibia and reducing anterior tibial translation and that the speed of this activation is vital for the subsequent joint stability (Shultz and Perrin, 1999). However, it should be noted that voluntary muscular control forms only part of the joint stabilisation process and should be explored alongside measures of intrinsic stiffening.

It is difficult to compare the findings of the current study to previous literature as to our knowledge this is the first study to examine the effect of fatigue on EMD during eccentric muscle actions of the hamstrings in elite female youth soccer players. A number of adult studies have investigated the effect of fatigue on EMD but mainly during isometric muscle actions and with adult populations. Previous literature identifies that EMD is influenced by: 1) the type of muscle action (Cavanagh and Komi, 1979); 2) joint angle (Grabiner, 1986); 3) the effort level (Vos et al. 1991); 4) fatigue (Nilsson et al. 1977); and 5) the age and sex of the participants (Clarkson and Kroll 1978). Previous studies have reported an increase in the EMD of the vastus lateralis muscle after fatiguing exercise ranging from 27%-57% (Zohu et al. 1996; Nilsson et al. 1977). The percentage change in EMD reported by Zhou et al. (1996) is similar to that reported in the current study (57% versus 58%) despite differences in the age group, sex, muscle tested and muscles action performed.
The limited studies reporting EMD data on children have been predominantly on boys, during isometric actions and in a non-fatigued state (Zohu et al. 1997; Falk et al. 2009; Cohen et al. 2010). Zhou et al. (1997) reported significantly longer EMD values in 8-12 year old children than in adults but no significant difference between EMD in the girls compared with the boys in the youngest age group (8-12 year olds). Others have found significantly longer EMD values in young boys and prepubertal girls compared with adults (Waugh et al. 2013; Cohen et al. 2010). The EMD data reported in the current study, albeit on the hamstrings during eccentric muscle actions, support this previously identified age related EMD difference. The U13 age group had a significantly longer EMD for all muscles tested (119 ms ± 49) compared with the U15 (93 ms ± 32) and U17s (92 ms ± 37) which may be related to maturation changes in the muscle-tendon ability to generate and transmit force. Differences such as lower muscle activation and lower muscle fibre conduction velocity have been implicated in this longer EMD displayed in children (Hanlin et al. 2003) and may increase the potential for injury. It is suggested that children recruit and utilise less type II muscle fibres during maximal voluntary muscle actions than adults and therefore show a longer EMD (Falk et al. 2009; Hanlin et al. 2003). More recently work by Waugh et al. (2013) have shown a direct relationship between muscle stiffness and EMD, whereby greater stiffness resulted in a shorter EMD. As leg stiffness appears to increase with chronological age (Lloyd et al. 2011) it is likely that muscle stiffness plays a part in age-related shortening of EMD. The data from the current study would appear to suggest that the influence of muscle stiffness driving EMD peaks and plateus at around 14 years of age and 1 year post peak height velocity in female youth footballers.

The current study is the first to demonstrate age-related effects in the EMD of the eccentric hamstrings when fatigue is present in female youth soccer players. The significantly longer EMD both pre and post fatigue displayed by the U13 age group indicates a reduced ability of younger girls to quickly activate their muscles and respond to a physical and visual stimulus. One possible explanation for this is that younger girls may have a more compliant muscle-tendon system compared with older girls
which requires more time to produce a mechanical response given the same stimulus (O’Brien et al. 2010; Kubo et al. 2001). The recent study of O’Brien et al. (2010) reported that tendon stiffness was 84% greater in women compared to young girls, mainly due to an increase in tendon cross-sectional area as opposed to tendon length. Another explanation for the age difference when fatigue is present could be due to the fibre type distribution, with type II fibres displaying shorter force-developing times compared to type I fibres (Winter and Brookes, 1991). Although muscle biopsy studies on children are sparse, the available literature indicates a decline in the percent of type I fibres from childhood to adolescence (Armstrong and Fawkner, 2008) which may partly explain the results seen in the current study, however this hypothesis requires further investigation. Research by Cohen et al. (2010) identified that the level of training did not have any significant effect on EMD in 9-12 year olds, when comparing EMD of endurance trained and untrained children. The findings of the current study may challenge this lack of training effect and suggest that the number of total hours of athlete exposure may influence EMD by reducing the detrimental effects of fatigue as shown in the U17 compared with the U13 age groups. This hypothesis does however require further investigation exploring the fatigue effects on EMD using non trained participants in each age group.

It would appear that the prolongation of EMD when fatigued maybe largely attributed to a failure somewhere in the muscle contraction process as EMD has been shown to increase in parallel with muscle fatigue (Horita and Ishiko, 1987). This does not however explain the significant age effect reported in the current study in the longer EMD both pre and post fatigue in the U13 age group when compared to the U15 and U17s. As the time it takes to stretch the series elastic component (SEC) forms a major part of EMD (Zohu et al. 1995, 1996; Winter and Brookes, 1991) the greater elasticity in muscle tissue in younger children could increase the time required to stretch the SEC and initiate a reflexive response (Zohu et al. 1995), and may account for the age related differences. The mechanisms involved in this increased EMD following fatigue in female youth soccer players could
be due to deterioration in muscle conductive, contractile or elastic properties and require further investigation.

The current study found no significant muscle specific differences in the EMD recorded pre or post the soccer specific fatigue protocol. These findings suggest that during eccentric hamstring actions in female youth soccer players, there are no differences in the feedback mechanisms between the medial and lateral hamstrings or the calf muscles. Padua el al. (2006) suggested that when fatigued female athletes show an ankle-dominant strategy when landing, with a greater reliance on the ankle musculature and less on the knee musculature. This shift from knee dominance pre fatigue to ankle dominance post fatigue to stabilise the joint is suggested to occur as the ankle muscles tend to be less fatigued. This shift to less fatigued musculature was seen as a compensatory mechanism in order to maintain leg stiffness when fatigued (Padua et al. 2006). The current findings suggest that following soccer specific fatigue, female youth soccer players show no differences in the feedback response of the hamstrings or the calf. This suggests that female youth soccer players do not move towards an ankle dominant strategy when fatigue is present, irrespective of age, maturational status or training status. This lack of a compensatory mechanism would suggest that female youth soccer players continue to rely on muscles that have reduced neuromuscular capability, and this may increase the risk of injury. However, a limitation of the current study, is that the role of the hip abductors/adductors or gluteal muscles was not explored. It is possible that the fatigue protocol used may have altered the EMD of the gluteals and/or hip abductors/adductors in order to compensate for fatigue. Additional research is necessary in order to investigate the effects of fatigue on the neuromuscular response of both the hip musculature and core muscles, and during differing muscle actions. Also as the hamstrings work concentrically during landing exploring fatigue related changes in EMD during concentric actions is warranted.
The current study has demonstrated significant acute age-related effects of a soccer specific fatigue and it is possible that neuromuscular feedback mechanisms remain compromised until the next training session, placing them at an increased relative risk of injury. A recent study by Howatson (2010) revealed that EMD remained compromised 96h following a fatigue task despite force production returning to pre fatigue levels at 48h. This has important implications for injury risk as neuromuscular feedback mechanisms appear to remain compromised even after muscular components have fully recovered. The residual effects of soccer specific fatigue on EMD requires further investigation as this data would give coaches vital information regarding the neuromuscular readiness of their players to re-perform, whilst identifying an optimal time to train to avoid increased relative risk of injury.

In conclusion, the current study is the first to identify fatigue related effects on neuromuscular control required to stabilise the joint in female youth soccer players. It should be noted that it was not the purpose of this study to determine joint stability but future work should aim to directly investigate fatigue related effects on joint stability in female youth soccer. Neuromuscular mechanisms have been shown to be compromised when muscular fatigue is present and therefore an emphasis of intervention programmes must be to develop neuromuscular functioning. Importantly these prevention programmes must include components that relate to fatigue resistance and should therefore be undertaken in the middle or towards the end of training sessions rather than solely in warm ups. The age-related effects found in the current study further reinforce that prevention strategies need to be embedded within training programmes from an early age.

**PERSPECTIVES**

The present study indicated that fatigue severely compromises neuromuscular control that is required to stabilise the knee in 13-17 year old elite female soccer players and that these effects are age specific with reduced neuromuscular function in younger children. Youth female players
do not appear to move from a knee to ankle dominant strategy to support joint control when fatigue is present. These data highlight that fatigue should be considered as a factor when devising injury screening procedures in female youth soccer players. Likewise, intervention programmes should begin in pre-pubery, should be age and maturation specific and focus on neuromuscular fatigue resistance, with the aim of improving neuromuscular feedback mechanisms.

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Figure 1: A diagrammatic representation of the SAFT\textsuperscript{90} course

Figure 2: Changes in combined EMD pre and post fatigue by age group (irrespective of muscle or movement velocity)

Contributorship:
All authors (MDSC, AP, RL, JO) contributed to the study design, interpretation of the data and drafting of the article. MDSC was responsible for the contextualisation of the study and the data analysis. MDSC and AP conducted the data collection.

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Competing Interests:
There are no competing interests in this study.