Received: 10 July 2020 | Revised: 22 September 2020 | Accepted: 24 September 2020

DOI: 10.1111/sms.13848

ORIGINAL ARTICLE

WILEY

Influence of sex and fiber type on the satellite cell pool in human skeletal muscle

Oscar Horwath[1] | **Marcus Moberg**[1] | **Filip J. Larsen**[1] | **Andrew Philp**[3,4] | **William Apró**[1,2] | **Björn Ekblom**[1]

[1]Department of Physiology, Nutrition and Biomechanics, Åstrand Laboratory, Swedish School of Sport and Health Sciences, Stockholm, Sweden

[2]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

[3]Diabetes and Metabolism Division, Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW, Australia

[4]St Vincent's Medical School, UNSW Medicine, UNSW Sydney, Sydney, Australia

Correspondence
Oscar Horwath, Department of Physiology, Nutrition and Biomechanics, Åstrand Laboratory, Swedish School of Sport and Health Sciences, Box 5626. SE 114 86 Stockholm, Sweden.
Email: oscar.horwath@gih.se

Funding information
Åke Wiberg Stiftelse, Grant/Award Number: M17-0259; The Swedish Military Research Authority, Grant/Award Number: AF 922 0916; H2020 European Research Council, Grant/Award Number: 707336; Stiftelsen Lars Hiertas Minne, Grant/Award Number: FO2017-0325; The Elisabeth and Gunnar Liljedahls Foundation; Centrum for Idrottsforskning, Grant/Award Number: D2017-0012, D2019-0050, P2018-0161, P2019-0061, P2019-0098, P2019-0193 and P2020-0058

The repair, remodeling, and regeneration of myofibers are dependent on satellite cells (SCs), although, the distribution of SCs in different fiber types of human muscle remains inconclusive. There is also a paucity of research comparing muscle fiber characteristics in a sex-specific manner. Therefore, the aim of this study was to investigate fiber type-specific SC content in men and women. Muscle biopsies from *vastus lateralis* were collected from 64 young (mean age 27 ± 5), moderately trained men (n = 34) and women (n = 30). SCs were identified by Pax7-staining together with immunofluorescent analyses of fiber type composition, fiber size, and myonuclei content. In a mixed population, comparable number of SCs was associated to type I and type II fibers (0.07 ± 0.02 vs 0.07 ± 0.02 SCs per fiber, respectively). However, unlike men, women displayed a fiber type-specific distribution, with SC content being lower in type II than type I fibers ($P = .041$). Sex-based differences were found specifically for type II fibers, where women displayed lower SC content compared to men ($P < .001$). In addition, positive correlations (r-values between 0.36-0.56) were found between SC content and type I and type II fiber size in men ($P = .03$ and $P < .01$, respectively), whereas similar relationships could not be detected in women. Sex-based differences were also noted for fiber type composition and fiber size, but not for myonuclei content. We hereby provide evidence for sex-based differences present at the myocellular level, which may have important implications when studying exercise- and training-induced myogenic responses in skeletal muscle.

KEYWORDS
fiber size, muscle plasticity, myonuclei, Pax7, vastus lateralis

1 | INTRODUCTION

Human skeletal muscle is a dynamic and highly plastic tissue, capable of altering its phenotype in response to divergent stimuli, such as chronic exercise or disuse.[1] The adaptability of muscle is in turn influenced by a number of different factors, such as sex,[2] age,[3] training history,[4] and fiber type composition.[5] Categorized according to myosin heavy chain

protein expression (MHC), human muscle consists of three primary muscle fiber types: type I, IIA, and IIX, each with distinct metabolic, contractile, and adaptive properties.[6,7] Investigations performed in a fiber type-specific manner are therefore valuable to uncover molecular aspects that advance our understanding of skeletal muscle physiology.

Muscle adaptation has been the subject of intense research during the last decades and increasing attention has been directed toward the role of satellite cells (SCs) in this process. SCs are a pool of undifferentiated stem cells involved in the repair, remodeling, and regeneration of muscle tissue.[8] Residing beneath the basal lamina, SCs are typically in a quiescent state, but can upon stimulation (eg, myofiber injury) progress through the myogenic pathway to repair segmental injuries or generate new myofibers.[9] SCs are also known mediators of exercise-induced adaptations as they can donate their nucleus to existing myofibers,[10] thereby supporting hypertrophy following resistance exercise.[11] Evidence suggests that SCs can contribute to myofiber remodeling also in the absence of fiber growth, such as in response to endurance-based exercise.[12,13]

So far, many studies have demonstrated that the SC pool is dynamic and capable of expanding in response to both acute and chronic exercise regimens;[11,14] however, the relative distribution of SCs in type I and type II fibers remains inconclusive. It has been shown that aging[3,15] and some clinical conditions[16,17] are accompanied by a reduced SC pool specifically in type II fibers, but this phenomenon has not been observed in young adults.[11,14] In fact, only one study has directly assessed whether SCs display a fiber type-specific distribution, reporting that no differences exist between slow and fast-twitch fibers.[18] It should, however, be noted that this analysis contained a small number of biological samples and that sex-specific information was lacking.

Muscle morphological disparities between men and women are well documented, where men typically display larger fiber areas and greater proportions of fast-twitch fibers than women.[19-22] However, there is a general lack of consensus whether the SC pool in human muscle is sex-dependent. Walker et al 2011 analyzed pooled samples from young and old individuals and found that women have a reduced SC pool compared to their male counterparts, but the apparent effect may have been accounted for by low SC content in old women.[20] In contrast, Kadi et al 2004 reported that SC content is similar in young, active men and women,[23] though, this study contained mixed-fiber analyses and samples were collected from *tibialis anterior* (TA), which in relation to *vastus lateralis* (VL) may have different fiber characteristics due to divergent functional demands.

Therefore, by applying immunohistochemical techniques, the aim of the present study was to investigate the SC distribution in type I and II muscle fibers of the VL muscle. In order to provide further insights into the SC pool of human muscle, we assessed potential differences between men and women. Based on previous data, we hypothesized that the VL muscle would be characterized by an equal SC distribution across fiber types. We further hypothesized that typical sex-based differences would be present in terms of fiber type composition and fiber size, that is, larger fiber areas and greater proportions of fast-twitch fibers in men, but not for SC content.

2 | MATERIAL AND METHODS

2.1 | Ethical approval

The present study involves secondary analyses performed on muscle samples collected in five ongoing projects with other specific research objectives. All studies have received ethical approval; study 1-4 from the Swedish Ethical Review Authority and study 5 from West Midlands—Black Country Research Ethics Committee. All studies are in agreement with the latest version of the Declaration of Helsinki, and an informed consent was obtained from all individuals prior to their participation.

2.2 | Participants

Thirty-four men and thirty women were included in the study. As mentioned above, they were originally recruited as participants in five ongoing studies, all with the overarching aim to study various muscle adaptations to different interventions. To be included in these studies, they had to be healthy and non-smoking, have a normal bodyweight and be between the ages of 18-40 years. They were also required to perform physical exercise on a regular basis. The specific inclusion criteria varied between the studies as follows: study 1) participants (n = 30) were required to perform endurance or resistance exercise, or both, at least 3 times per week, study 2) participants (n = 9) were required to perform resistance exercise 2-3 times per week, where one session per week had to involve exercises for the lower body, study 3) participants (n = 10) were required to perform both resistance- and endurance-type exercise at least 2 times per week, with no upper limit for exercise participation study 4) participants (n = 5) were required to perform endurance exercise at least 3 times per week, without the inclusion of high-intensity interval training, study 5) participants (n = 10) were required to be physically active up to 3 times per week but not involved in any structured resistance exercise program. The endurance-type exercise reported by the participants included running, cycling, swimming, and team sports. The resistance-type exercise included weight training and CrossFit. Based on these criteria, all participants were considered moderately trained.

Overall, the spectrum of exercise modes was similar between men and women. Physical characteristics of the participants are presented in Table 1.

2.3 | Muscle biopsies

The muscle biopsy was obtained after an overnight fast, and the participants were instructed to refrain from alcohol, caffeine, and vigorous physical exercise for 48 hours before tissue sampling. Following administration of local anesthesia (Carbocaine 20 mg mL^{-1}, AstraZeneca AB, Sweden), one sample was taken from the VL muscle with a Weil-Blakesley conchotome, or with the Bergstrom needle adapted for manual suction, both techniques described in detail here.[24] After quickly removing excess blood, fiber bundles were mounted in OCT-embedding medium (Tissue-Tek OCT compound) and frozen in isopentane cooled by liquid nitrogen. These samples were then stored at −80°C until sectioning commenced.

2.4 | Immunohistochemistry

Muscle biopsies were cut to 7 μm thick cross-sections using a cryostat (Leica CM1950), placed on microscope glass slide (VistaVision, VWR International), air-dried at room temperature, and later stored in −80°C. Immunohistochemical procedures to determine fiber type composition, fiber size, and SC and myonuclei content have previously been described in detail elsewhere.[5] Briefly, for fiber typing, unfixed slides were first blocked for 60 minutes (1% NGS and fat-free milk), and then incubated overnight with a primary antibody against laminin (1:50; D18, Developmental Studies Hybridoma Bank (DSHB), USA). The next day, after being washed in PBS, slides were incubated for 60 minutes with primary antibodies against MHC isoform proteins; MHC-I (1:500; BA-F8, DSHB, USA) and MHC-II (1:250; SC-71, DSHB, USA). Following PBS washes, slides were incubated with secondary antibodies (1:100; Goat anti-mouse 350 IgG2A, 1:500; 488 Goat anti-mouse IgG2B and 1:500; 594 Goat anti-mouse IgG1, Alexa Fluor, Invitrogen, USA), before being mounted with Prolong Gold Antifade Reagent (Invitrogen, USA). For SCs and myonuclei, slides were first fixed (4% paraformaldehyde) for 20 minutes, washed in PBS, then blocked for 30 minutes (1% BSA and 0.01% Triton X-100). Following blocking, slides were incubated overnight using a cocktail of primary antibodies against laminin (1:200; D18, DSHB, USA), MHC-I (1:500; BA-F8, DSHB, USA), and Pax7 (1:100; 199010, Abcam, GBR). The next day, after washes in PBS, slides were incubated with secondary antibodies (1:500; 488 Goat anti-mouse IgG2A, 1:2000; 488 Goat anti-mouse IgG2B and 1:500; 594 Goat anti-mouse IgG1, Alexa Fluor, Invitrogen, USA), and mounted with Prolong Gold Antifade Reagent containing DAPI (Invitrogen, USA) to permit labeling of nuclei.

TABLE 1 Participant characteristics

	Men (n = 34)	Women (n = 30)
Age (years)	27 ± 5 (19-40)	28 ± 4 (20-35)
Body mass (kg)	79 ± 8 (64-98)*	66 ± 7 (55-81)
Height (cm)	179 ± 6 (169-195)*	168 ± 5 (159-179)
BMI	25 ± 2 (22-29)*	23 ± 2 (19-27)

Note: Values are means ± SD (range).

Abbreviation: BMI; body mass index.

*Significantly different from women ($P < .01$).

2.5 | Image acquisition and analysis

Stained sections were captured using a widefield fluorescent microscope (Celena S, Logos Biosystems, South Korea). Digital pictures were processed using image analysis software (Celena S Digital Imaging System, Logos Biosystems, South Korea), and analyses were carried out using ImageJ (National Institutes of Health, USA). Fiber type composition was determined by counting the number of each fiber type on the whole muscle section (average of 773 ± 339 fibers per biopsy included for this analysis) and their relative abundance was expressed as percentage of total fiber number. The present study did not differentiate between MHC-IIA and IIX fibers as preliminary data revealed that pure MHC-IIX fibers represented ~2-3% of the total fiber population, which is too low to provide accurate results. Likewise, the abundance of fibers expressing more than one MHC isoform protein (ie, hybrid fibers) accounted for <2% of total fiber counts and was therefore omitted from the analysis. Furthermore, the MHC-stained slides were also used for determining fiber type-specific area (fCSA), in which the laminin border of individual fibers was manually encircled, including 50 fibers/fiber type for analysis. Fibers that were damaged or longitudinally oriented were not considered in the analysis and the form factor was applied to ensure fiber circularity in muscle cross-sections[25] (type I 0.87 and type II 0.87 vs. type I 0.87 and type II 0.88, in men and women, respectively). In addition, the percentage fiber type area (fCSA %) was calculated as showed in the example; mean type I fiber area (μm^2) multiplied by proportion of type I fibers (%) divided by total fiber area (μm^2). This measure reflects the proportion of the muscle cross-section occupied by one particular fiber type. Moreover, Pax7-positive cells ($Pax7^+$) situated inside the laminin border were in case of co-localization with DAPI considered SCs. These cells were then marked together with their associated fiber type, either type I (MHC-I positive) or type II (MHC-I negative). A representative picture of this staining is provided in Figure 1 and

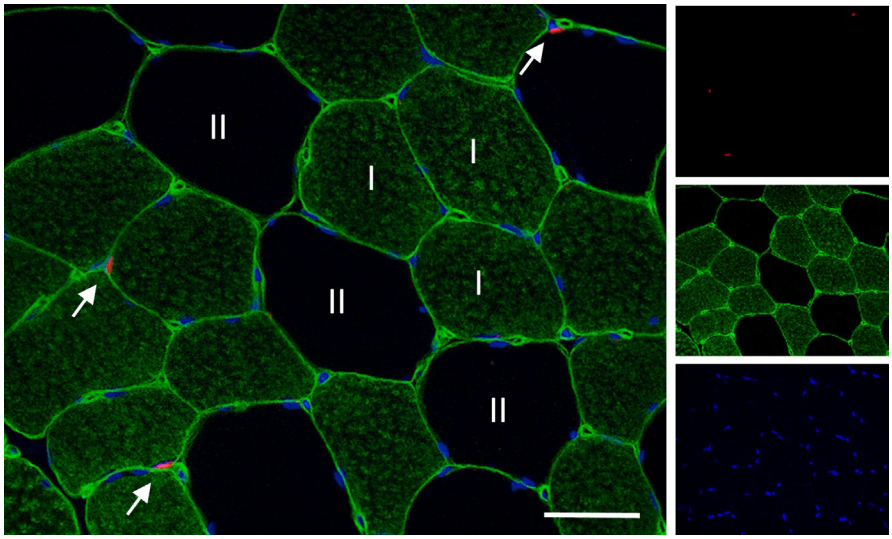

FIGURE 1 Representative picture of fiber type-specific SC staining in muscle cross-section. Merged image of Pax7, laminin, MHC type I fibers and DAPI-stained myonuclei (left panel). Single channel Pax7 (top right panel). Single channel laminin and MHC-I (middle right panel). Single channel DAPI (bottom right panel). Arrows indicate SCs (red cells in the periphery of the fiber) and I and II represent type I and type II muscle fibers, respectively. Scale bar = 50 μm

an average of 370 ± 119 fibers per biopsy were included for analysis (158 ± 66 and 212 ± 78 type I and type II muscle fibers, respectively). The same slides were then used to determine myonuclei content, where each nucleus had to have more than half of its geometrical center located within the laminin border to be considered a myonucleus ($DAPI^+/Pax7^-$). Myonuclei were after enumeration expressed per fiber and in relation to fiber area (ie, myonuclear domain). This analysis contained 50 fibers per fiber type per biopsy.[26]

2.6 | Statistical analysis

All data are expressed as means ± SD. Participants and muscle fiber characteristics (mixed population) were analyzed with independent *t* tests. Fiber type-specific variables in men and women were analyzed using a two-way analysis of variance (ANOVA) with factors for sex (men vs. women) and fiber type (type I vs. type II). In case of significant interaction, sex- or fiber type-specific differences were followed up with Bonferroni´s multiple comparisons test. Correlations between fiber area and SC and myonuclei content were analyzed with Pearson product moment correlation coefficient (*r*). All analyses were performed in GraphPad Prism (version 8.4.2 for Windows; GraphPad Software). Statistical significance was accepted at $P < .05$.

3 | RESULTS

3.1 | Participant characteristics

Participant characteristics are provided in Table 1. Age was similar between groups, whereas typical sex-based differences were observed for body mass, height, and BMI ($P < .01$).

3.2 | Muscle fiber characteristics in a mixed population

Including both men and women, the VL muscle contained a greater proportion of type II fibers than type I fibers (56 ± 10% vs. 44 ± 10%, respectively; $P < .001$) (Table 2). The mean type II fiber area was greater than the type I fiber area (+11%, $P = .014$), and type II fCSA (%) was superior to the corresponding type I fCSA (%) (+38%, $P < .001$). The number of SCs per fiber did not show a fiber type-specific distribution, nor did SCs per myonuclei, although, when SC content was related to fiber size, type II fibers had a lower

TABLE 2 Muscle fiber characteristics in a mixed population

	Type I	Type II
FT composition (%)	44 ± 10 (17-66)	56 ± 10* (33-83)
fCSA (μm^2)	5260 ± 1135 (3177-8475)	5864 ± 1558* (3562-11900)
fCSA (%)	42 ± 11 (12-65)	58 ± 11* (35-88)
SCs per fiber	0.07 ± 0.02 (0.03-0.14)	0.07 ± 0.02 (0.04-0.14)
SCs per myonuclei (%)	2.8 ± 0.8 (0.9-5.3)	2.5 ± 0.8 (0.9-4.7)
SCs per mm^2 area	13.6 ± 3.7 (5.0-27.5)	11.3 ± 3.5* (4.4-25.3)
Myonuclei per fiber	2.5 ± 0.4 (1.7-3-7)	2.5 ± 0.4 (1.8-4.0)
Myonuclear domain	2146 ± 287 (1343-2984)	2305 ± 444* (1447-3094)

Note: Values as means ± SD (range).

Abbreviations: fCSA, fiber cross-sectional area; fCSA %, percentage fiber type area; FT, fiber type; SCs, satellite cells. Myonuclear domain; myonucleus per μm^2.

*Significantly different from type I fibers ($P < .05$).

TABLE 3 Muscle fiber characteristics in men and women

	Men		Women	
	Type I	**Type II**	**Type I**	**Type II**
FT composition (%)	41 ± 11[a] (17-63)	59 ± 11[ab] (37-83)	47 ± 9 (23-66)	53 ± 9[b] (34-77)
fCSA (μm^2)	5708 ± 1200[a] (3177-8475)	6785 ± 1292[ab] (4971-11900)	4752 ± 810 (3561-7078)	4820 ± 1118 (3562-8612)
fCSA (%)	37 ± 11[a] (12-59)	63 ± 11 [ab] (41-88)	46 ± 9 (20-65)	54 ± 9 [b] (35-80)
SCs per fiber	0.07 ± 0.02 (0.03-0.14)	0.08 ± 0.02[a] (0.05-0.14)	0.07 ± 0.02 (0.04-0.10)	0.05 ± 0.02[b] (0.02-0.11)
SCs per myonuclei (%)	3.0 ± 0.8[cd] (0.9-5.3)	2.8 ± 0.7[c] (1.8-4.7)	2.6 ± 0.7[d] (1.5-4.7)	2.2 ± 0.8 (0.9-4.0)
SCs per mm^2 area	13.1 ± 3.6[d] (5.0-20.5)	11.2 ± 2.4 (7.9-17.3)	14.1 ± 3.9[d] (7.2-27.5)	11.5 ± 4.5 (4.4-25.2)
Myonuclei per fiber	2.4 ± 0.4 (1.7-3.4)	2.6 ± 0.4 (2.0-4.0)	2.5 ± 0.5 (1.8-3.7)	2.5 ± 0.5 (1.8-4.0)
Myonuclear domain	2369 ± 352[a] (1589-2984)	2627 ± 297[ab] (1883-3094)	1892 ± 243 (1343-2409)	1941 ± 265 (1447-2499)

Note: Values are means ± SD (range).

Abbreviations: FT, fiber type; fCSA, fiber cross-sectional area; fCSA %, percentage fiber type area; SCs, satellite cells. Myonuclear domain; myonucleus per μm^2.

[a]Significantly different from corresponding fiber type in women (sex x fiber type interaction, $P < .05$).

[b]Significantly different from type I fibers (within group, $P < .05$).

[c]Significant main effect of sex ($P < .05$).

[d]Significant main effect of fiber type ($P < .05$).

number of SCs per mm^2 area than type I fibers (−17%, $P < .001$). Furthermore, myonuclei per fiber was similar across fiber types, but when related to fiber size, type II fibers had greater myonuclear domains compared to type I fibers (+7%, $P < .032$).

3.3 | Muscle fiber characteristics in men and women

Muscle fiber characteristics in men and women are presented in Table 3. Sex-based differences were found for FT composition and fiber size, where men had a greater proportion of type II fibers ($P = .045$), and greater type I and type II mean fiber areas compared to women (+20% and +40%, respectively; $P < .01$). Whereas men had superior type II compared to type I fiber areas (+18%, $P < .001$), the fiber types were of similar size in women. In addition, type II fCSA (%) was greater than the corresponding type I fCSA (%) in both sexes; however, this effect was even more pronounced in men than in women ($P < .001$). The number of SCs per fiber was different between sexes, where men had greater SC content in type II fibers, but not type I fibers, compared to women (+60%, $P < .001$) (Table 3 and Figure 2). In men, SCs were equally distributed across fiber types, whereas women displayed lower SC content in the type II fibers (−28%, $P = .041$). No

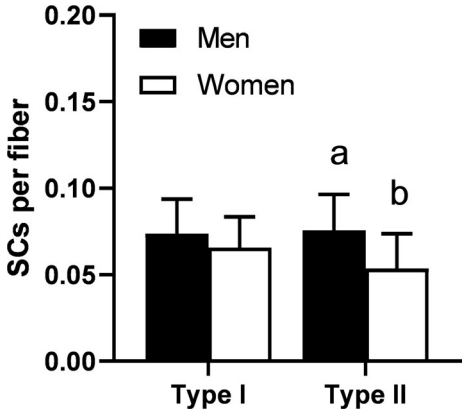

FIGURE 2 Fiber type-specific SC content in men and women. [a]Significantly different from corresponding fiber type in women (sex x fiber type interaction, $P < .05$). [b]Significantly different from type I fibers (within group, $P < .05$)

sex-based differences were observed for any other SC-related variable, although SCs per myonuclei and SCs per mm^2 area displayed an effect of fiber type ($P = .049$ and $P < .001$, respectively) and SCs per myonuclei showed an effect of sex ($P < .001$). The number of myonuclei per fiber was equal across sex and fiber type; however, sex-based differences were found for myonuclear domain ($P = .048$), where men had greater values in both type I and type II fibers compared

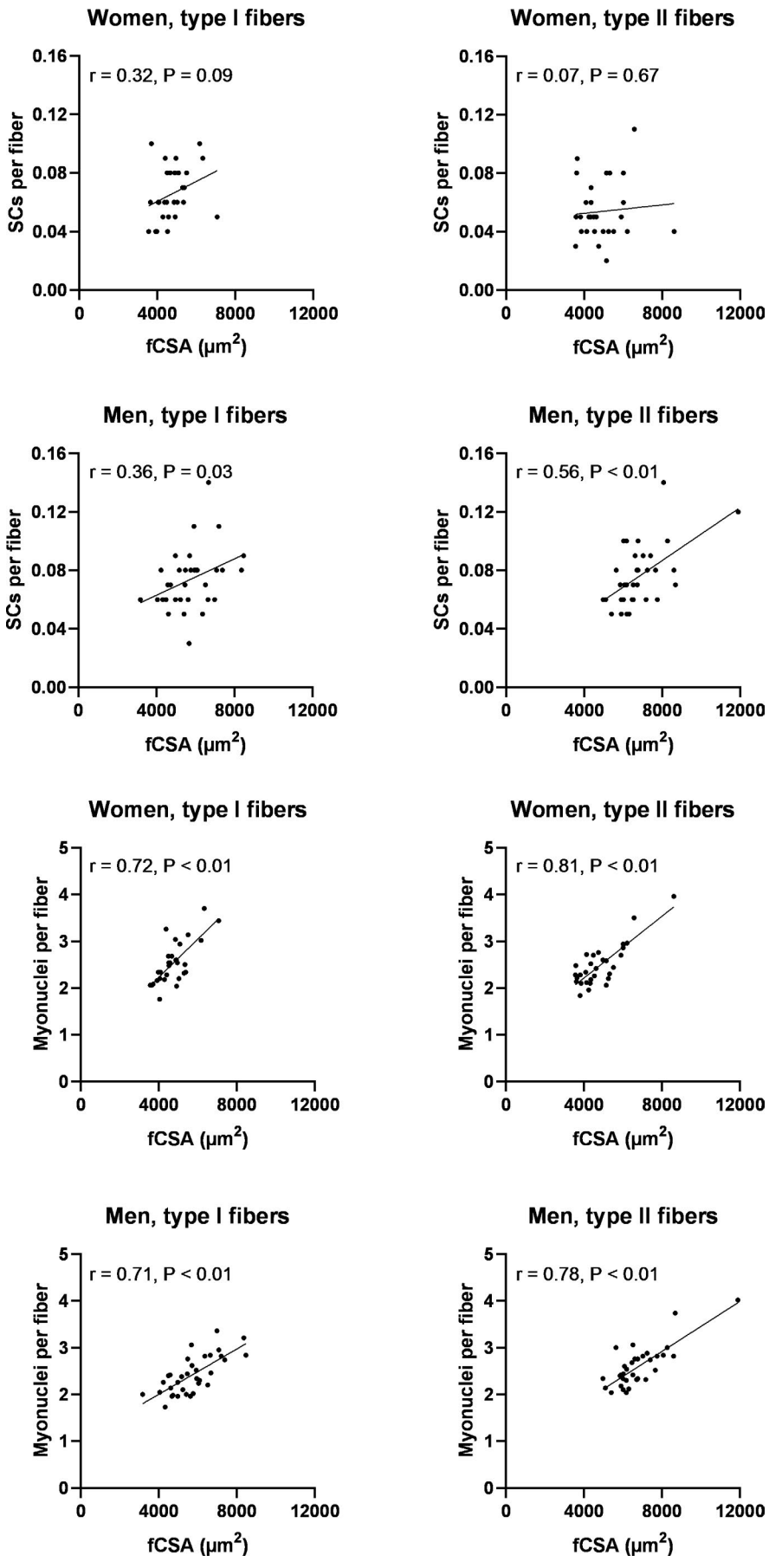

FIGURE 3 Relationship between fiber area and SC- and myonuclei content in men and women. Correlational analyses of SC content and fiber area (top four panels) in type I fibers (left panels) and type II fibers (right panels). Correlational analyses of myonuclei content and fiber area (bottom four panels) in type I fibers (left panels) and type II fibers (right panels). Corresponding *r* and *P* values are shown for each relationship

to women (+25% and +35%, respectively; $P < .001$). The myonuclear domains were of equal size in women, but not in men, where type II fibers were superior (+10%, $P < .001$) (Table 3).

3.4 | Correlational analyses

Correlational analyses between fiber area and SC and myonuclei content in men and women are presented in Figure 3. Significant correlations were found between fiber size and SC content in type I and type II fibers for men ($r = 0.36$, $P = .032$ and $r = 0.56$, $P < .001$, respectively), but not for women. Furthermore, men and women displayed significant correlations between fiber area and myonuclei content in both fiber types ($P < .001$), see Figure 3 (bottom four panels).

4 | DISCUSSION

This morphological study showed that SCs are not distributed in a fiber type-specific manner in the VL muscle of a large number of moderately trained individuals. However, sex-based analyses revealed that SCs were differentially expressed in type I and type II fibers in women, and SC content was lower compared to men specifically in the type II fibers. In addition, SC content was associated to myofiber size in men, but not in women. These new findings may have implications for future studies investigating myogenic adaptations to exercise and training.

The VL muscle is commonly described with mixed-fiber type composition.[21] However, inherent variation exists between and within individuals,[27] and taking into account sex,[19,21] and training history,[28] this notion should be treated cautiously. Indeed, the present study showed that trained individuals have a greater proportion of type II than type I fibers (56% vs. 44%, respectively), although, the inclusion of participants engaged in various types of exercise modes precludes any conclusions whether a certain type of exercise may have contributed to the current phenotype. Importantly, the apparent deviation from a mixed-fiber type composition was further accentuated when expressed as fCSA (%), particularly evident in men (>60% type II fibers). Given that fCSA (%) correlates well with MHC content,[29] a differential fiber type expression of this magnitude should be considered in analyses of muscle homogenates, where the signal obtained is thought to reflect a mixture of both fiber types. However, for some samples, such as those with high MHC-II content, this may not be accurate as the response would likely be representative of the larger number of type II fibers. As such, this may confound data interpretation in studies where large differences in MHC composition exist between cohorts (eg, trained vs untrained). This highlights the advantage of performing fiber type-dependent analyses, for instance, through isolation of individual muscle fibers.[7] However, from a practical perspective, the already time-consuming process of fiber isolation may require more manual labor when dissecting samples with a skewed fiber type composition.

The present study reported greater type II fiber proportion in men compared to women, previously observed in untrained,[19,21] and trained individuals,[30] as well as in other skeletal muscles.[22] This sexual dimorphism may, at least partly, be accounted for by circulating hormones as both testosterone, estrogen and thyroid hormone can impact muscle fiber composition.[31] Furthermore, sex-based differences have also been identified at the transcriptional level, in the form of mRNA levels of the MHC-encoding genes, but not at the protein level.[30] The fact that mRNA transcripts and corresponding protein products were divergent may suggest that MHC gene expression is regulated differently in men and women by a post-translational mechanism (eg, microRNAs). Evidence in mice suggests that microRNAs can modulate skeletal muscle phenotype,[32] though, this is yet to be demonstrated in human muscle and requires further research.

In line with previous literature,[19,21,22] the present study pointed out typical sex-related differences in fiber size, where men displayed superior fCSA irrespective of fiber type. Nonetheless, unlike in men, women demonstrated equal type I and type II fiber sizes, a finding that opposes prior work in women of similar age, who generally manifested greater type I fiber sizes.[19,21] As these reports included primarily untrained subjects, discrepancies between reports are likely due to differences in training status. More specifically, the current study included women who regularly performed endurance and resistance exercise, where the latter is known to stimulate type II fiber hypertrophy,[33] thus causing a more balanced fiber size profile.

Many laboratories have studied the SC pool in the context of muscle plasticity over the last decades. For instance, Kadi et al (2006) studied the SC pool in the VL muscle of physically active adults and found that SCs were distributed independently of muscle fiber type.[18] Nevertheless, due to a limited sample size (n = 5), sex-specific analyses were not provided and the authors speculated that a greater number of observations might have altered their conclusion. Thus, in order to properly revaluate this hypothesis, the present study extended previous work by assessing a greater number of biopsy samples (n = 64), including ~24 000 muscle fibers and ~1500 SCs in the analysis. The findings were, however, comparable to those described in Kadi et al (2006), whereby a similar number of SCs were associated to type I and type II fibers (0.07 ± 0.02 vs. 0.07 ± 0.02 SCs per fiber, respectively), indicating that SC content is relatively constant across studies in the basal state of young active adults. Of note, the present study and others have previously reported

SC content in the range of 0.05-0.10 SCs per fiber with the Pax7-antibody,[20,28,34] whereas some presented markedly higher counts using the same marker (0.15-0.20 SCs per fiber).[12] Whether this is due to differences in study populations, methodological/analytical procedures, time of biopsy sampling or simply reflective of the heterogeneity in skeletal muscle remains undefined.

Some reports have performed mixed-fiber analyses of young subjects in age-related comparisons,[20,23,35] although, SC content has not previously been investigated in a sex- and fiber type-specific manner in young, trained adults. Also, there is currently limited data regarding the SC pool in women as the majority of studies conducted at the myocellular level are carried out in men.[8,10] The present study found that women displayed fewer SCs associated with type II than type I fibers. In addition, women had lower type II fiber SC content than men (0.05 ± 0.02 vs. 0.08 ± 0.02 SCs per fiber, respectively), an effect present also when SCs were related to myonuclei content. Despite that the functional implications from our findings require further research, it could be speculated that a lower SC availability may have negative effects on muscle adaptation, as demonstrated by the concomitant reduction in SC content and muscle mass during aging.[3] Similarly, Petrella et al (2008) showed that greater basal SC abundance, and thereby a higher capacity for myonuclear accretion, was associated with more pronounced hypertrophy after resistance training.[11] In contrast, myalgic women undergoing resistance training displayed an inverse relationship between basal SC content and fiber size changes.[17] Thus, based on these studies, it is difficult to determine the relative importance of the basal SC pool in mediating muscle adaptation to exercise. Nonetheless, we and others have previously reported high responsiveness in women´s type II fibers to anabolic stimulus,[5,33] which in turn would indicate that other factors than basal SC abundance, such as the ability to activate the SC pool,[14] might have greater impact on muscle trainability. Future studies should investigate if a relatively low SC availability may impede long-term training responses in healthy adults, and if so, determine strategies to induce SC pool expansion to promote optimal conditions for muscle adaptation.

To further delineate the role of SCs in myofiber size regulation, SCs were expressed in relation to fCSA, whereby it was observed that the previously obtained sex-based differences for type II fibers (SCs per fiber) were no longer present when expressed as SC density (SCs per mm^2 area). This finding could therefore be explained by relatively smaller fiber sizes in women, rather than inherent differences in the SC pool. However, our rather weak but positive correlations (*r*-values between 0.36-0.56 for type I and type II fibers, respectively) may indicate that a relationship exists between SC content and fiber size in men, but not in women. So far, the exact mechanism for this occurrence remain undefined, but one could speculate that hormonal regulation plays a role since circulating androgen levels affect both fiber areas and SC and myonuclei content in human muscle,[5,36] yet this cannot explain the lack of association in women. We would instead argue that the positive correlations found are explained by greater mean fiber sizes in men, for which the dependency of the SC pool increases to mediate further hypertrophy,[11] but also to aid in the repair and remodeling of myofibers that comes with daily wear and tear. This reasoning is in line with prior work showing a similar relationship in trained, but not in untrained muscle,[28] providing supporting evidence that larger myofibers may require a greater SC pool. Some reports have also suggested that SC content can predict myofiber size in an aging population.[37] Based on the present data, we would therefore argue that the size of the SC pool is associated to myofiber size, which in turn is largely influenced, but not limited to biological sex.

Lastly, even if no sex-dependent differences were observed for myonuclei content, clear correlations were found between fiber size and myonuclei content (*r*-values between 0.71-0.81), providing support for the myonuclear domain theory, which continues to be widely debated topic within the field of muscle biology.[38] On the other hand, we noted that men had myonuclear domains (~2300 and 2600 μm^2 for type I and type II fibers, respectively) that were at, or even above the suggested limit for what a single myonucleus can regulate in terms of transcriptional capacity (~2250 μm^2).[35] This would in turn partly argue against the concept of "ceiling theory" where additional myonuclei are required to sustain transcriptional output once a certain threshold has been surpassed. Although the reason for these high myonuclear domains is unclear, it may indicate that our enumeration process of myonuclei was more restrictive than reported elsewhere,[35] (ie, $DAPI^+$ cells were considered myonuclei if their geometric center was clearly inside of the laminin border), resulting in relatively lower myonuclei counts. However, we used established criteria for determining myonuclei content in human muscle and therefore finds this unlikely.[26,39] We rather suggest that the myonuclear domain is more flexible than originally proposed and that the theoretical limit for when additional myonuclei are required can be surpassed in trained individuals, particularly in type II fibers, as discussed elsewhere.[38] Furthermore, similar to findings presented by other research groups,[35] our results show that women have significantly smaller myonuclear domains (ie, higher myonuclear density) than men. Although the biological relevance of an elevated myonuclear density is presently unknown, this may provide a potential explanation for the lower SC content in women as a greater transcriptional capacity per area would indicate that the requirement for SC-mediated addition of new myonuclei is low. Nonetheless, these sex-based differences are thus important to consider when studying the regulatory mechanisms that underpin muscle hypertrophy

in similar populations. For example, as women are further away from their transcriptional maximum, it could be hypothesized that women are less likely to incorporate additional myonuclei in response to given hypertrophic stimuli, whereas men would be more dependent on myonuclear accretion to facilitate myofiber growth at an early stage. This is in line with retrospectively analyzed data presented here,[40] demonstrating that myofibers with large myonuclear domains (>2000 μm^2) at baseline increased their myonuclei content to a greater extent than fibers with small myonuclear domains (<1700 μm^2); however, the hypertrophic response was similar in both groups, indicating that other mechanism beside myonuclear addition determines myofiber growth.

Due to the nature of the current investigation, in which secondary analyses were performed on samples collected in different research projects, there is a lack of detailed information regarding the participant´s exercise regimens. We acknowledge that such data would have excluded any possibilities that results were confounded by variations in exercise habits (eg, movement patterns, intensity, volume, and duration); however, based on our inclusion criteria, we believe that the participants accurately reflects a trained population which allowed for fair comparisons between sex- and fiber type-specific variables.

In conclusion, the main finding of the present study was that SCs were not associated to a particular fiber type in the basal state of trained individuals. However, sex-specific analyses revealed that women had a fiber type-specific SC distribution and lower SC content specifically in type II fibers, as compared to men. This study also demonstrates a relationship between SC content and fiber size in men, whereas this finding could not be detected in women. These data therefore collectively highlight sex-specific differences at the myocellular level that may have implications for future research when studying myogenic adaptations to exercise and training.

5 | PERSPECTIVE

The present study provides insights in basal muscle fiber characteristics and highlights important sex-based differences at the myocellular level. Here, it was found that SCs are not associated to a particular fiber type in a mixed population. However, women demonstrated a fiber type-specific SC distribution and had lower SC content compared to men specifically in the type II fibers. These findings may therefore have implications for future studies, for example, the relatively lower SC content in women´s type II fibers and greater myonuclear domains may increase their propensity for SC pool expansion and myonuclear addition in response to a hypertrophic stimulus. Further studies are although required to delineate if these findings could be relevant to explain potential sex-based differences in muscle adaptation. Given the importance of SCs in muscle plasticity, it should also be investigated whether a relatively low SC content is associated with impaired adaptive capacities, and in that case, determine applicable countermeasures, such as exercise and/or nutritional strategies in order to ensure optimal skeletal muscle function.

ACKNOWLEDGEMENTS

The authors would like to thank all the volunteers for their participation in this study.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

ORCID

Oscar Horwath https://orcid.org/0000-0002-3500-2896
Marcus Moberg https://orcid.org/0000-0003-3747-0148
Filip J. Larsen https://orcid.org/0000-0002-1343-8656
Andrew Philp https://orcid.org/0000-0003-3860-4136
William Apró https://orcid.org/0000-0003-1942-2919
Björn Ekblom https://orcid.org/0000-0002-4030-5437

REFERENCES

1. Blaauw B, Schiaffino S, Reggiani C. Mechanisms modulating skeletal muscle phenotype. *Compr Physiol*. 2013;3(4):1645-1687.
2. Esbjornsson Liljedahl M, Holm I, Sylven C, Jansson E. Different responses of skeletal muscle following sprint training in men and women. *Eur J Appl Physiol Occup Physiol*. 1996;74(4):375-383.
3. Verdijk LB, Koopman R, Schaart G, Meijer K, Savelberg HH, van Loon LJ. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am J Physiol Endocrinol Metab*. 2007;292(1):E151-E157.
4. Moberg M, Lindholm ME, Reitzner SM, Ekblom B, Sundberg CJ, Psilander N. Exercise induces different molecular responses in trained and untrained human muscle. *Med Sci Sports Exerc*. 2020;52(8):1679-1690.
5. Horwath O, Apro W, Moberg M, et al. Fiber type-specific hypertrophy and increased capillarization in skeletal muscle following testosterone administration of young women. *J Appl Physiol*. 2020;128(5):1240-1250.
6. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev*. 2011;91(4):1447-1531.
7. Edman S, Soderlund K, Moberg M, Apro W, Blomstrand E. mTORC1 signaling in individual human muscle fibers following resistance exercise in combination with intake of essential amino acids. *Front Nutr*. 2019;6:96.
8. Snijders T, Nederveen JP, McKay BR, et al. Satellite cells in human skeletal muscle plasticity. *Front Physiol*. 2015;6:283.
9. Lepper C, Partridge TA, Fan CM. An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. *Development*. 2011;138(17):3639-3646.
10. Shamim B, Hawley JA, Camera DM. Protein availability and satellite cell dynamics in skeletal muscle. *Sports Med*. 2018;48(6):1329-1343.

11. Petrella JK, Kim JS, Mayhew DL, Cross JM, Bamman MM. Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: a cluster analysis. *J Appl Physiol*. 2008;104(6):1736-1742.
12. Joanisse S, Gillen JB, Bellamy LM, et al. Evidence for the contribution of muscle stem cells to nonhypertrophic skeletal muscle remodeling in humans. *FASEB J*. 2013;27(11):4596-4605.
13. Joanisse S, McKay BR, Nederveen JP, et al. Satellite cell activity, without expansion, after nonhypertrophic stimuli. *Am J Physiol Regul Integr Comp Physiol*. 2015;309(9):R1101-R1111.
14. Bellamy LM, Joanisse S, Grubb A, et al. The acute satellite cell response and skeletal muscle hypertrophy following resistance training. *PLoS ONE*. 2014;9(10):e109739.
15. Verdijk LB, Snijders T, Drost M, Delhaas T, Kadi F, van Loon LJ. Satellite cells in human skeletal muscle; from birth to old age. *Age (Dordr)*. 2014;36(2):545-547.
16. Snijders T, Verdijk LB, Hansen D, Dendale P, van Loon LJ. Continuous endurance-type exercise training does not modulate satellite cell content in obese type 2 diabetes patients. *Muscle Nerve*. 2011;43(3):393-401.
17. Mackey AL, Andersen LL, Frandsen U, Sjogaard G. Strength training increases the size of the satellite cell pool in type I and II fibres of chronically painful trapezius muscle in females. *J Physiol*. 2011;589(Pt 22):5503-5515.
18. Kadi F, Charifi N, Henriksson J. The number of satellite cells in slow and fast fibres from human vastus lateralis muscle. *Histochem Cell Biol*. 2006;126(1):83-87.
19. Staron RS, Hagerman FC, Hikida RS, et al. Fiber type composition of the vastus lateralis muscle of young men and women. *J Histochem Cytochem*. 2000;48(5):623-629.
20. Walker DK, Fry CS, Drummond MJ, et al. PAX7+ satellite cells in young and older adults following resistance exercise. *Muscle Nerve*. 2012;46(1):51-59.
21. Simoneau JA, Bouchard C. Human variation in skeletal muscle fiber-type proportion and enzyme activities. *Am J Physiol*. 1989;257(4 Pt 1):E567-E572.
22. Miller AE, MacDougall JD, Tarnopolsky MA, Sale DG. Gender differences in strength and muscle fiber characteristics. *Eur J Appl Physiol Occup Physiol*. 1993;66(3):254-262.
23. Kadi F, Charifi N, Denis C, Lexell J. Satellite cells and myonuclei in young and elderly women and men. *Muscle Nerve*. 2004;29(1):120-127.
24. Ekblom B. The muscle biopsy technique. Historical and methodological considerations. *Scand J Med Sci Sports*. 2017;27(5):458-461.
25. Charifi N, Kadi F, Feasson L, Costes F, Geyssant A, Denis C. Enhancement of microvessel tortuosity in the vastus lateralis muscle of old men in response to endurance training. *J Physiol*. 2004;554(Pt 2):559-569.
26. Mackey AL, Kjaer M, Charifi N, et al. Assessment of satellite cell number and activity status in human skeletal muscle biopsies. *Muscle Nerve*. 2009;40(3):455-465.
27. Blomstrand E, Ekblom B. The needle biopsy technique for fibre type determination in human skeletal muscle—a methodological study. *Acta Physiol Scand*. 1982;116(4):437-442.
28. Mackey AL, Karlsen A, Couppé C, et al. Differential satellite cell density of type I and II fibres with lifelong endurance running in old men. *Acta Physiol (Oxf)*. 2014;210(3):612-627.
29. Fry AC, Allemeier CA, Staron RS. Correlation between percentage fiber type area and myosin heavy chain content in human skeletal muscle. *Eur J Appl Physiol Occup Physiol*. 1994;68(3):246-251.
30. Maher AC, Fu MH, Isfort RJ, Varbanov AR, Qu XA, Tarnopolsky MA. Sex differences in global mRNA content of human skeletal muscle. *PLoS ONE*. 2009;4(7):e6335.
31. Haizlip KM, Harrison BC, Leinwand LA. Sex-based differences in skeletal muscle kinetics and fiber-type composition. *Physiology (Bethesda)*. 2015;30(1):30-39.
32. McCarthy JJ, Esser KA. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J Appl Physiol*. 2007;102(1):306-313.
33. Staron RS, Malicky ES, Leonardi MJ, Falkel JE, Hagerman FC, Dudley GA. Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *Eur J Appl Physiol Occup Physiol*. 1990;60(1):71-79.
34. Damas F, Libardi CA, Ugrinowitsch C, et al. Early- and later-phases satellite cell responses and myonuclear content with resistance training in young men. *PLoS ONE*. 2018;13(1):e0191039.
35. Petrella JK, Kim JS, Cross JM, Kosek DJ, Bamman MM. Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *Am J Physiol Endocrinol Metab*. 2006;291(5):E937-E946.
36. Sinha-Hikim I, Roth SM, Lee MI, Bhasin S. Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *Am J Physiol Endocrinol Metab*. 2003;285(1):E197-E205.
37. Verdijk LB, Snijders T, Beelen M, et al. Characteristics of muscle fiber type are predictive of skeletal muscle mass and strength in elderly men. *J Am Geriatr Soc*. 2010;58(11):2069-2075.
38. Murach KA, Englund DA, Dupont-Versteegden EE, McCarthy JJ, Peterson CA. Myonuclear domain flexibility challenges rigid assumptions on satellite cell contribution to skeletal muscle fiber hypertrophy. *Front Physiol*. 2018;9:635.
39. Psilander N, Eftestøl E, Cumming KT, et al. Effects of training, detraining, and retraining on strength, hypertrophy, and myonuclear number in human skeletal muscle. *J Appl Physiol*. 2019;126(6):1636-1645.
40. Snijders T, Aussieker T, Holwerda A, Parise G, van Loon LJC, Verdijk LB. The concept of skeletal muscle memory: Evidence from animal and human studies. *Acta Physiol (Oxf)*. 2020;229(3):e13465.

How to cite this article: Horwath O, Moberg M, Larsen FJ, Philp A, Apro W, Ekblom B. Influence of sex and fiber type on the satellite cell pool in human skeletal muscle. *Scand J Med Sci Sport*. 2020;00:1–10. https://doi.org/10.1111/sms.13848