The influence of anti-inflammatory medication on exercise-induced myogenic precursor cell responses in humans

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1Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark; 2Department of Physical Education and Health, Orebro University, Orebro, Sweden; 3Department of Immunology and Microbiology, University of Southern Denmark, Odense; and 4Department of Pathology, Odense University Hospital, Odense, Denmark

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Mackey AL, Kjaer M, Dandanell S, Mikkelsen KH, Holm L, Døssing S, Kadi F, Koskinen SO, Jensen CH, Schroder HD, Langberg H. The influence of anti-inflammatory medication on exercise-induced myogenic precursor cell responses in humans. J Appl Physiol 103: 425–431, 2007. First published April 26, 2007; doi:10.1152/japplphysiol.00157.2007.—The consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) is widespread among athletes when faced with muscle soreness or injury, but the effects of NSAIDs on satellite cell activity in humans are unknown. To investigate this, 14 healthy male endurance athletes (mean peak oxygen consumption 62 ml·kg⁻¹·min⁻¹) volunteered for the study, which involved running 36 km. They were divided into two groups and received either 100 mg indomethacin per day or placebo. Muscle biopsies collected before the run and on days 1, 3, and 8 afterward were analyzed for satellite cells by immunohistochemistry with the aid of neural cell adhesion molecule (NCAM) and fetal antigen-1 (FA1) antibodies. Muscle biopsies were also collected from untrained individuals for comparison. Compared with preexercise levels, a 27% increase in the number of NCAM+ cells was observed on day 8 postexercise in the placebo group (P < 0.05), while levels remained similar at all time points in the NSAID group. No change was seen in the proportion of FA1+ cells, although lower levels were found in the muscle of endurance-trained athletes compared with untrained individuals (P < 0.05). These results suggest that ingestion of anti-inflammatory drugs attenuates the exercise-induced increase in satellite cell number, supporting the role of the cyclooxygenase pathway in satellite cell activity.

SATELLITE CELLS are crucial for skeletal muscle adaptation to exercise. They contribute to hypertrophy by providing new myonuclei and repair damaged segments of mature myofibers for successful regeneration following injury or exercise-induced muscle damage (13–15, 40). While there is ample evidence to show that exercise can stimulate satellite cells to re-enter the cell cycle and proliferate, reviewed by Kadi et al. (20), the mechanisms by which this activation occurs are not yet fully understood (3). It has been reported that cyclooxygenase (COX) is necessary for satellite cell activity (31) and muscle regeneration (2, 4), and furthermore, it has been shown that inhibition of COX activity by ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) suppresses the increased mixed muscle protein synthesis rates normally observed following exercise (46). Such an observation is clinically relevant given the widespread consumption of NSAIDs among athletes (27, 48).

There are many reports of the effects of different types of exercise on satellite cells in old and young individuals of different training status (summarized in Table 1). Studies examining the effects of a single bout of exercise involving high force eccentric muscle contractions, for example, have revealed significant increases in the number of satellite cells shortly after exercise (9, 10). Regular repeated training over longer periods has also been investigated and, while similar numbers of satellite cells were reported with 8–16 wk of strength training (16), other studies have found significant increases in satellite cell number in both young and old individuals (6, 21, 26, 35, 38). As can be seen from the summary of these studies in Table 1, a single bout of maximal eccentric contractions on an isokinetic dynamometer induces a much stronger satellite cell response than a period of regular strength training, possibly reflecting the requirement of satellite cell activity in the processes of regeneration and hypertrophy, respectively. There is, however, a lack of information on the effects of a single bout of exercise in well-trained athletes, as all of the previously mentioned studies involved untrained subjects for whom the exercise was unaccustomed. With this in mind, the present study was designed to examine the influence of NSAIDs on satellite cells and their response to exercise in well-trained humans.

A secondary aim of the present study was to compare two markers of satellite cells, as there are many different markers currently in use but no consensus as to which is the most reliable. Neural cell adhesion molecule (NCAM; also known as CD56 or Leu19) has been used in many studies to identify satellite cells (see Table 1). Another potential marker of satellite cells is fetal antigen-1 (FA1), whose larger membrane-associated precursor is known as human delta-like (dlk). FA1 is a member of the growth factor superfamily and believed to be a marker of cells with regenerative potential (12). While increased numbers of FA1+ cells were observed in skeletal muscle biopsies in response to a single bout of high-intensity exercise involving eccentric contractions (9), the significance of FA1 staining of satellite cells remains unclear. To our knowledge there have not yet been any studies investigating

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Summary of recent human studies investigating the response of satellite cells to exercise

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<tbody>
<tr>
<td>Single biopsy</td>
<td>Elite power lifters vs. controls</td>
<td>NCAM</td>
<td>~70% more satellite cells in</td>
<td>Kadi et al. (23)</td>
</tr>
<tr>
<td></td>
<td>High-level power lifters</td>
<td>NCAM</td>
<td>power lifters than controls</td>
<td>Eriksson et al. (11)</td>
</tr>
<tr>
<td>Single bout: eccentric exercise</td>
<td>Untrained young and old men</td>
<td>NCAM</td>
<td>↑141% (young), ↑51% (old)</td>
<td>Dreyer et al. (10)</td>
</tr>
<tr>
<td>Single bout: eccentric exercise</td>
<td>Untrained young men</td>
<td>NCAM</td>
<td>↑192% 4 days postexercise</td>
<td>Cramer et al. (9)</td>
</tr>
<tr>
<td>Single bout: 36-km run</td>
<td>Endurance-trained young men</td>
<td>NCAM</td>
<td>↑27% 8 days postexercise</td>
<td>Present study</td>
</tr>
<tr>
<td>9-wk Strength training</td>
<td>Active old men</td>
<td>NCAM</td>
<td>↑29%</td>
<td>Charifi et al. (6)</td>
</tr>
<tr>
<td></td>
<td>Young and old men and women</td>
<td>EM</td>
<td>↑55%</td>
<td>Roth et al. (38)</td>
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<tr>
<td>10-wk Strength training</td>
<td>Young women</td>
<td>NCAM</td>
<td>↑46%</td>
<td>Kadi and Thornell (22)</td>
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<tr>
<td>12-wk Strength training</td>
<td>Untrained old men and women</td>
<td>NCAM</td>
<td>↑27%</td>
<td>Mackey et al. (26)</td>
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<tr>
<td>14-wk Strength training</td>
<td>Untrained young men</td>
<td>NCAM</td>
<td>↑31%</td>
<td>Kadi et al. (21)</td>
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<tr>
<td>16-wk Strength training</td>
<td>Untrained young men</td>
<td>NCAM</td>
<td>↑ further enhanced with creatine</td>
<td>Olsen et al. (34)</td>
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<td></td>
<td>Untrained young and old men and</td>
<td>NCAM</td>
<td>supplementation</td>
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<tr>
<td>8- or 16-wk Strength training</td>
<td>women</td>
<td>EM</td>
<td>↑ in young men only</td>
<td>Petrella et al. (35)</td>
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Analysis was carried out by immunostaining of muscle biopsy cross sections with an anti-neural cell adhesion molecule (NCAM) antibody or by electron microscopy (EM). Percent changes presented are based on values presented in the original articles, where satellite cell number is expressed relative to fiber number or number of nuclei.

the extent of colocalization of NCAM and FA1, and this study therefore examined the colocalization of these two proteins in muscle biopsies obtained from trained and untrained individuals and also assessed the influence of exercise on this colocalization. We hypothesized that 36-km running in trained individuals would influence the satellite cell pool and that the ingestion of NSAIDs would alter this response. With regard to FA1 and NCAM colocalization, our hypothesis was that trained and untrained individuals would display different staining patterns.

METHODS

Volunteers and exercise. Fourteen endurance-trained athletes [mean age 25 ± 3 (SD) yr; height 1.83 ± 0.06 m; weight 79 ± 9 kg; peak oxygen consumption (\(\text{VO}_2\) peak) 62 ± 6 ml·kg\(^{-1}\)·min\(^{-1}\)] volunteered for the study, which involved running a 36-km race and the collection of muscle biopsies before the race and on days 1, 3, and 8 afterward. One of the criteria for participation in the study was that the individual had not taken NSAID during the 6 mo before the study. The subjects were randomly assigned to two groups: one group received NSAID (100 mg indomethacin per day) from 4 days before the run until the final biopsy was collected, while the second group received a placebo over the same period. To avoid any effect of NSAID ingestion on baseline measurements, the prebiopsy was collected at least 7 days before the run. Subjects were asked to refrain from physical exercise for 10 days before the run. Muscle biopsies were also collected from a second group of 12 healthy untrained males [mean age 25 ± 3 (SD) yr; height 1.83 ± 0.06 m, and weight 81 ± 14 kg] as a control group for the FA1 staining analysis. All participants gave written informed consent for the study, which was approved by the Ethics Committees of the Municipalities of Copenhagen and Frederiksberg (Ref no. KF 01-164/97) and conformed to the Declaration of Helsinki.

Muscle biopsies. Muscle biopsies were obtained from the midportion of vastus lateralis muscle using a standard needle biopsy technique. For the individuals involved in the 36-km run, four biopsies in total were collected from each person. Biopsies were taken from alternate legs, the second biopsy from each leg being taken ~5 cm away from the incision site of the first biopsy. On extraction, the sample was mounted and frozen by immersion in isopentane precooled to approximately −160°C by liquid nitrogen. Samples were stored at −80°C pending analyses. Serial transverse sections (10 μm) were cut at −24°C using a cryostat and mounted on SuperFrost Plus slides (Menzel-Gläser, Braunschweig, Germany).

Immunohistochemistry. Satellite cells were visualized by immunohistochemical staining using a mouse anti-human NCAM antibody (CD56, cat. no. 347740; Becton Dickinson, San Jose, CA), following a previously described protocol (6). Counting was carried out on 842 ± 396 fibers (mean ± SD), 227 being the minimum number of fibers in any of the biopsies examined. Values were expressed relative to the number of myonuclei [NCAM+/cells/(myonuclei + NCAM+ cells) × 100].

To examine NCAM/FA1 colocalization, double staining was carried out on two serial transverse sections (10 μm) from each biopsy. The first section was stained with mouse anti-human NCAM and rabbit anti-human FA1 (developed at University of Southern Denmark, Odense, Denmark) antibodies to examine the extent of colocalization. The second section was stained for merosin (Clone Mer3/22B2; Novocastra, Newcastle upon Tyne, UK) and FA1 to establish the location of the FA1+ cells. Merosin is a muscle-specific laminin located in the basement membrane (41). Double staining with merosin and FA1 allows determination of whether the cells are located within the fibers or relative to the cell membranes. Merosin was preincubated 1 h at room temperature in blocking solution (1% bovine serum albumin, 1% goat serum, and 0.5% triton X-100). Secondary antibody bound to FITC was added and incubated for 1 h at room temperature. The slides were then washed extensively in PBS and mounted in antifade Hoechst 33342 (Molecular Probes cat. no. H11001) solution. A cover slip was then added at a 45° angle to minimize effects on the existing fluorescence.
Table 2. Satellite cell counts from vastus lateralis muscle obtained before and 24 h, 3 days, and 8 days after a 36-km run from individuals who either consumed placebo or NSAID

<table>
<thead>
<tr>
<th></th>
<th>Preexercise</th>
<th>24 h</th>
<th>3 Day</th>
<th>8 Day</th>
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<tr>
<td>Total NCAM+</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>4.10 ± 0.86</td>
<td>4.14 ± 0.32</td>
<td>4.97 ± 0.21</td>
<td>5.14 ± 1.08*</td>
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<tr>
<td>NSAID</td>
<td>4.21 ± 0.97</td>
<td>3.84 ± 0.62</td>
<td>4.49 ± 0.74</td>
<td>4.07 ± 0.86</td>
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<tr>
<td>FA1+ and NCAM+</td>
<td></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>2.31 ± 1.73</td>
<td>2.68 ± 1.69</td>
<td>2.37 ± 1.30</td>
<td>2.85 ± 1.96</td>
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<tr>
<td>NSAID</td>
<td>2.89 ± 0.96</td>
<td>2.51 ± 1.27</td>
<td>3.31 ± 2.11</td>
<td>2.03 ± 1.39</td>
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<tr>
<td>Central nuclei fibers, %</td>
<td></td>
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<tr>
<td>Placebo</td>
<td>0.51 ± 0.27</td>
<td>1.05 ± 0.78</td>
<td>1.35 ± 2.18</td>
<td>1.69 ± 1.88</td>
</tr>
<tr>
<td>NSAID</td>
<td>1.04 ± 1.12</td>
<td>1.24 ± 0.69</td>
<td>1.06 ± 0.74</td>
<td>1.37 ± 1.60</td>
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<tr>
<td>Embryonic myosin+ fibers, %</td>
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<tr>
<td>Placebo</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.05</td>
<td>0.17 ± 0.22</td>
</tr>
<tr>
<td>NSAID</td>
<td>0.01 ± 0.02</td>
<td>0.07 ± 0.12</td>
<td>0.01 ± 0.04</td>
<td>0.02 ± 0.04</td>
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Values are means ± SD; n = 5 subjects who consumed placebo and 5 subjects who consumed nonsteroidal anti-inflammatory drug (NSAID). Satellite cell values are presented in 2 ways: as the number of NCAM+ cells (total NCAM+) and as the number of cells positive for both NCAM and fetal antigen-1 (FA1+ and NCAM+), both expressed relative to myonuclear number. The percentage of fibers that contained centrally located nuclei or cytoplasmic staining for embryonic myosin is also included. Values are means ± SD. *Significantly different from preexercise values, P < 0.05.

**RESULTS**

Placebo and NSAID groups were similar with regard to age, height, weight, and VO2peak and completed the 36-km running in similar times: 160 ± 23 and 163 ± 17 min, respectively. Of the seven subjects in each group, a full set of muscle biopsies (preexercise, 24 h, 3 day, and 8 day) was analyzed for six subjects in the placebo group and five subjects in the NSAID group. The exclusion of three subjects was due to either a subject missing a biopsy time point or poor quality of one or more of the biopsies obtained from a subject.

**Satellite cells**

Satellite cell data expressed relative to myonuclear number are displayed in Table 2. Statistical analysis of these data using the Kolmogorov-Smirnov normality test revealed nonnormal distribution of total NCAM+ data for the 8-day time point in the NSAID group. To confirm the data to a Gaussian distribution, the values were expressed relative to preexercise values, which passed the normality test. These adjusted data are presented in Fig. 2. Two-way repeated-measures ANOVA analysis of the adjusted data revealed no interaction between the placebo and NSAID groups but an overall significance for time (P = 0.016). One-way repeated-measures ANOVA analysis showed a significant difference in the placebo group (P = 0.014), while no change was observed in the NSAID group (P = 0.384). Post hoc testing of the placebo group data identified day 8 as being significantly different from preexercise values (P < 0.05), increasing by 27% from 4.10 ± 0.86 to 5.14 ± 1.08 on day 8. Repeated-measures ANOVA analysis of
the numbers of NCAM+ and FA1+ cells presented in Table 2 showed similar levels at each time point.

**FA1 staining.** The FA1 staining pattern was a fine delineation of the cell border, similar to NCAM staining, and from the NCAM/FA1 double staining, it was apparent that the staining patterns of these two antibodies were almost identical: differences could only be observed at ×60 magnification or higher. Some cells stained very intensely, but these were rare. Figure 1 shows staining images from double staining with NCAM and FA1, and merosin and FA1. On analysis of the relative proportion of FA1+ cells with the 36-km run, no effect was observed over time (see Table 2). Figure 3 displays the proportion of NCAM+ cells stained by FA1, showing clearly that untrained individuals have a higher proportion than endurance-trained individuals (73 ± 12% compared with 48 ± 18%, respectively). Of all cells counted (either positive for NCAM alone, FA1 alone, or both), 19 ± 15% were positive for FA1 only. This value was identical for both trained and untrained groups. To find out more about these cells, double staining for merosin and FA1 was carried out, and this allowed us to determine the proportion of FA1+ cells located inside or outside the basement membrane. In the untrained group, 26 ± 21% of all FA1 cells were found to be located outside the merosin-defined muscle borders compared with 35 ± 22% in the trained group (not significant). Correlations between the location of FA1+ cells and the NCAM/FA1 double-staining results are presented in Fig. 4 and show a strong positive relationship between FA1+ cells that are located outside merosin-defined muscle borders and cells that are positive for FA1 but negative for NCAM. Similarly, those cells that are positive for both NCAM and FA1 demonstrate a strong positive relationship with the FA1+ cells located inside merosin-defined muscle borders.

**Regeneration.** The number of fibers positive for embryonic myosin was assessed for each section, and the result is expressed as a percentage of the total number of fibers in the section. The results are presented in Table 2, and the values ranged from 0 to 0.52%. Of the total of 16 embryonic+ fibers observed from all the sections studied, 5 of those demonstrated normal dystrophin staining, while the dystrophin staining around the other 11 fibers was either very faint or completely absent. The number of fibers with centrally located nuclei (see Table 2) ranged from 0 to 6%. There was no significant change with time or interaction between the placebo and NSAID.
groups for either the percentage of embryonic+ fibers or the percentage of fibers with central nuclei.

DISCUSSION

The new findings presented in this study reveal increased numbers of NCAM-stained cells in response to a 36-km run, suggesting that satellite cells were activated to reenter the cell cycle and proliferate with the exercise. While enhancement of the satellite cell pool has been demonstrated in several previous studies, this is, to our knowledge, the first study to show a response in individuals who are highly trained and accustomed to the exercise intervention. We also report here, for the first time, data suggesting that ingestion of NSAIDs may attenuate this response (Fig. 2), providing in vivo support for a role for the COX pathway in satellite cell activity in humans. While some authors have reported no effect of NSAIDs on satellite cell activity or muscle regeneration (45), several studies have demonstrated a negative effect of NSAID administration on various stages of myogenesis and regeneration (2, 4, 31, 33, 49, 50). The results of those experiments are supported by the present study, where no increase in the number of satellite cells, as assessed by NCAM staining, was observed in response to exercise in individuals who consumed NSAIDs.

The role of NSAIDS in satellite cell activity. Evidence for inflammation as a catalyst for activation of satellite cells comes from the many studies that have investigated the effects of blocking inflammation, directly on satellite cells in vitro, and also in animal models in vivo. Several other factors have also been shown to be capable of activating satellite cells, including hepatocyte growth factor (HGF) (1), nitric oxide synthase (43), and the insulin-like growth factor-1 (IGF-1) isoform known as mechanogrowth factor (17), while the IGF-1 isoform IGF-1Ea, along with other growth factors, acts to enhance proliferation (36). The precise mechanisms by which these agents activate satellite cells are not fully understood, but there is evidence for some specific interactions. For example, it is known that HGF acts on satellite cells via c-met, the HGF receptor expressed on quiescent satellite cells (8). Similarly, the role for inflammatory cells in satellite cell activation comes from the observation that quiescent murine satellite cells also express VCAM-1, whose coreceptor is found on infiltrating leukocytes (19). The influence of COX activity on myoblasts is through the conversion of arachidonic acid to prostaglandins (PGs), which have been shown to be important in muscle development (30), the different PG subtypes being implicated in the different stages of myoblast fusion (18). In the present study, COX-blocking NSAIDs were given in amounts known to block PG synthesis, and it has previously been demonstrated with the use of microdialysis that NSAID administration resulted in a complete drop in PGE_2 interstitial tissue concentration (25). Thus the present data support the view that COX-induced PG synthesis is important for satellite cell activity.

PGs serve many physiological functions in skeletal muscle, such as contributing to the regulation of blood flow (5, 32), and it is possible that this effect in itself prevented satellite cells from responding to the stimulus in the NSAID group, since this would reduce the transport of other signaling agents to and from the muscle. Although blood flow was not monitored in the present study, and it is difficult to comment on the extent of this effect, other data have shown that PG alone did not influence blood flow in skeletal muscle during exercise (24). While the likely function of NSAIDs is in the prevention of PG synthesis through COX inhibition, it is also known that different NSAIDs can act by various COX-independent mechanisms, inhibiting some pathways and activating others (44). We can therefore not rule out the possibility that pathways other than the COX/PG pathway have contributed to the attenuating effect of NSAIDs on satellite cell activity observed in the present study. However, the lack of any increase in satellite cell number with NSAIDs suggests that the presence of PG is important for this response.

FA1 staining. Another finding of the present study was that FA1 stained 25% more satellite cells (NCAM+) in healthy untrained individuals compared with trained individuals (Fig. 3). This is, to our knowledge, the first study that has quantitatively investigated the colocalization of FA1 and NCAM. It has been suggested that FA1 is a marker of cells with regenerative potential (12). With this in mind, together with the theory that adult skeletal muscle might contain different subpopulations of myogenic precursor cells, it is plausible that FA1 identifies a subpopulation of myogenic precursor cells in human skeletal muscle. This theory is supported by the fact that some of these cells were observed to occupy the classic satellite cell position under the basal lamina, whereas others were located outside the muscle fiber. While the majority of FA1+ cells were also NCAM+, 19% of all cells counted were FA1 + NCAM−, and strong correlations between these data and the merosin/FA1 staining data suggest that the FA1+ NCAM− cells were located outside the fibers (see Fig. 4). At this point of the discussion it should be remembered that markers for identification of myogenic precursor cells continue to be explored and validated, in particular in adult human skeletal muscle during events induced by exercise. Furthermore, it is possible that expression of NCAM on myogenic precursor cells may not be constant but could for example be conditional on their location or activation status. While we cannot rule out the possibility that FA1 is expressed in other cell types such as fibroblasts and inflammatory cells, it is possible that these FA1+ NCAM− cells are a myogenic precursor cell subpopulation that is either 1) migrating into the muscle fiber or 2) leaving it. In addition, although it is not possible to determine the exact origin and destination of these cells at this point, the presence of FA1+ cells inside and outside the muscle cell is an interesting finding, and that regular training influences the proportion of NCAM+ FA1+ cells supports the theory that FA1 labels a subpopulation of myogenic precursor cells. With regard to why trained athletes have a lower proportion of these cells compared with untrained individuals, it is tempting to speculate that this is indicative of a lower remaining regenerative potential in the trained muscle. The basis for this speculation is that it has been suggested that FA1 is present in cells with regenerative potential (12), and furthermore that it appears the proliferative potential of satellite cells is limited (39); thus the many cycles of damage and repair induced over many years of training would result in a lower remaining capacity for cell division. However, as the significance of FA1 staining remains to be clarified, it is not possible to comment conclusively on these findings at this time.

Satellite cells and exercise. This is the first study we are aware of that has examined the influence of a single bout of endurance running on satellite cells in trained individuals. The
numbers of satellite cells at baseline were similar to those in our untrained group and agree with satellite cell numbers in young men at rest reported in some studies (21, 23, 34, 35), although others have reported lower resting values (9, 10, 16, 37, 47). In some cases this discrepancy is likely to be due to the different methods of detection used, i.e., electron microscopy vs. immunohistochemistry, or the use of different antibodies, e.g., Pax7 vs. NCAM. It would not be unexpected to find higher numbers of satellite cells in endurance-trained individuals, since Charifi et al. (6) reported increased satellite cell numbers following a period of endurance training. However, based on the similar levels of satellite cells (using an NCAM antibody) observed in the untrained subjects in the present study and elsewhere (21, 23, 34, 35), it appears that long-term endurance training (~5 yr) results in levels of satellite cells comparable to untrained active individuals.

The unchanged levels of the proportion of FA1+ cells with 36-km running in the present study are in contrast to the results reported by Crameri et al. (9), where significantly higher levels of FA1+ cells were observed on days 4 and 8 postexercise. The two major differences between these two studies were the training status of the participants and the exercise interventions employed. The subjects in the present study were well-trained endurance runners who completed a single bout of exercise to which they were already accustomed, whereas the untrained volunteers in the study by Crameri et al. (9) performed one bout of high-intensity eccentric exercise, which is likely to have resulted in a much greater insult to the muscle. These differences may also explain the difference between the two studies in the magnitude of response of NCAM+ cells. Crameri et al. (9) reported an increase of almost 200% in the number of NCAM+ cells on day 4 postexercise, an increase that was still evident by day 8. In the present study, the modest 27% increase in the placebo group was not observed until day 8 postexercise and is more in line with previous studies involving periods of training lasting several months (6, 21, 26, 38). One obvious question concerns the purpose of the new satellite cells reported in such studies. In adult muscle, one of the main functions of satellite cells is to provide new myonuclei for maintenance and growth. No increased numbers of myonuclei were observed in the present study, however, and it is unlikely that enhancement of the satellite cell pool following a single bout of exercise can be explained by replacing existing myonuclei, since this occurs at a very low rate (42). Another function of satellite cells is to produce new myofibers to fuse with existing fibers to repair areas of damage induced by injury or exercise. Healthy skeletal muscle can regenerate fully following exercise-induced damage, and this process would be a reason for activation of satellite cells. Altered patterns of regeneration, as assessed by embryonic myosin staining, have been reported with NSAID administration following contraction-induced injury in rabbit skeletal muscle (33). Regeneration was therefore also investigated in the present study, and while some embryonic myosin-positive and dystrophin-negative fibers, as well as central nuclei, were observed, no statistically significant effect of either exercise or NSAID ingestion could be detected for these parameters. Furthermore, no sign of muscle damage in the form of necrotic muscle fibers was observed. It is likely that this is because the exercise was something the subjects were accustomed to. While some studies have reported no increase in inflammation in skeletal muscle with exercise (29), or increased inflammation following multiple biopsies alone (28), there is evidence for acute and chronic inflammatory responses in human skeletal muscle following certain types of exercise, especially where eccentric contractions are involved [reviewed by Clarkson and Hubal (7)]. Despite the uncustomed high-force muscle contractions performed in the study by Crameri et al. (9), evidence of necrosis was only observed in one of eight subjects, which taken together with the results of the present study suggests that satellite cells do not require muscle damage (as evidenced by visible signs of necrosis at the light microscopy level) to be stimulated to proliferate. Furthermore, it appears that the extent of proliferation is influenced by the type of exercise and how well the subjects are accustomed to it, a factor that is affected by the prior training status of the individual. While the purpose of the new satellite cells remains to be determined, it is possible that they simply return to a quiescent state in the absence of subsequent stimuli.

In conclusion, given the vital role satellite cells play in skeletal muscle maintenance and regeneration, any factors that interfere with their activity could negate the anabolic effects exerted by exercise, and while the precise mechanism by which the COX pathway acts on satellite cells and the ensuing events in the repair or hypertrophy process remains unclear, there is mounting evidence for a positive regulatory effect on satellite cell activity. This is relevant for the vast number of athletes who consume NSAIDs to continue training when faced with muscle soreness or injury, and also for the various disease conditions, such as osteoarthritis, where NSAIDs are prescribed. In conclusion, the data from this study suggest a negative regulatory effect of NSAIDs on the number of NCAM+ cells in human muscle, supporting the view that the COX pathway is important for normal satellite cell activity.

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