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Exercise and leukocyte interchange among central circulation, lung, spleen, and muscle ☆

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Abstract

Circulating leukocytes increase rapidly with exercise then quickly decrease when the exercise ends. We tested whether exercise acutely led to bidirectional interchange of leukocytes between the circulation and the lung, spleen, and active skeletal muscle. To accomplish this it was necessary to label a large number of immune cells (granulocytes, monocytes, and lymphocytes) in a way that resulted in minimal perturbation of cell function. Rats were injected intravenously with a single bolus of carboxyfluorescein diacetate succinamidyl ester (CFSE) dye which is rapidly and irreversibly taken up by circulating cells. The time course of the disappearance of labeled cells and their reappearance in the circulation following exercise was determined via flow cytometry. The majority of circulating leukocytes were labeled at 4 h. post-injection and this proportion slowly declined out to 120 h. At both 24 and 120 h, running resulted in an increase in the proportion of labeled leukocytes in the circulation. Analysis of the skeletal muscle, spleen and lung indicated that labeled leukocytes had accumulated in those tissues and were mobilized to the circulation in response to exercise. This indicates that there is an ongoing exchange of leukocytes between the circulation and tissues and that exercise can stimulate their redistribution. Exchange was slower with muscle than with spleen and lung, but in all cases, influenced by exercise. Exercise bouts redistribute leukocytes between the circulation and the lung, spleen and muscle. The modulatory effects of exercise on the immune system may be regulated in part by the systemic redistribution of immune cells.

Keywords

Lymphocyte; Monocyte; Neutrophil; Trafficking; Running

1. Introduction

In humans and rodents, circulating leukocytes (namely, neutrophils, lymphocytes, and monocytes) increase rapidly with brief exercise then quickly decrease when the exercise ends (Shephard, 2003). Little is known about the dynamic, bidirectional interchange of

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immune cells between the circulating blood and accessible pools or compartments. Some leukocytes reside within the vascular space but adhere to the endothelium (Dimitrov et al., 2010). The concept of leukocyte "homing" in which immune cells from one compartment can relocate to another in response to specific stresses has been observed with exercise (Kruger and Mooren, 2007) and relevant compartments include the lung (O'Dea et al., 2009; Schwab et al., 2003; van Teijlingen et al., 2003) and the spleen (Kamei and Carman, 2010). In addition, there is a growing body of data from air pollution to surgery, that *local* triggers can influence *systemic* immune cell function (den Hartigh et al., 2010; Landis, 2009; Menzies et al., 2006; Thomas et al., 2002). More recently, investigators have focused on exercising or injured muscle as a destination for circulating immune cells, and there is a growing appreciation for the potential role that immune cells play in muscle growth and repair related to exercise (Brigitte et al., 2010; Koh and Pizza, 2009; Tidball and Villalta, 2010).

We hypothesized that exercise would lead to a bidirectional flow of leukocytes between the circulation and the lung, spleen, and muscle. Our hypothesis contrasts with the current understanding of leukocyte trafficking that accompanies other perturbations, for example, infection or frank sepsis in which profound activation of leukocytes and accompanying endothelial damage typically results in immune cell egress from the circulating pool, diapedesis, and, ultimately, apoptosis (Fielding et al., 2008; Guo et al., 2002; Pero et al., 2007; Razavi et al., 2004; Speyer et al., 2004). While exercise leads to some degree of leukocyte activation, the bulk of evidence indicates that the degree of priming is substantially more subtle in exercise than occurs with trauma or sepsis (Pyne et al., 2000; Radom-Aizik et al., 2008). Moreover, while similarities exist between exercise and other forms of stress, most prominently, through the release of catecholamines into the circulation, exercise is a unique physiological perturbation in that it also leads to increased cardiac output and vascular shear forces, enhanced circulation to working muscle, and substantial changes in pH and temperature, all factors that can, independently, influence leukocyte trafficking and function (Beste and Hammer, 2008; Choi et al., 2008; Nash et al., 2008; Skilbeck et al., 2001).

The emerging view of circulating leukocytes (including innate immune cells like neutrophils) is that beyond their role in the control of foreign pathogens they are also responsive to growth factors and cytokines, and can contribute to angiogenesis and tissue repair (Seta and Kuwana, 2007; Tazzyman et al., 2009; Tidball and Wehling-Henricks, 2007)—all factors key to the adaptation to exercise. We also know that the exercise associated increase in leukocyte number is accompanied by alterations in leukocyte function and gene expression, the latter reflected by changes in both mRNA and microRNA (Radom-Aizik et al., 2009a,b, 2008, 2010). The pattern of ingress and egress of leukocytes associated with exercise is poorly understood – information that would be essential to determine the role cell trafficking in the adaptation to physical activity.

Most previous studies of leukocyte trafficking *in vivo* involve removal of circulating cells, labeling with radioactive tracers, and then reintroducing the cells into the circulation (MacIntyre et al., 2000; Paulsen et al., 2010). Leukocytes can then be traced at the level of the whole body, or, alternatively, by direct examination of tissue biopsies or samples. Such

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procedures have certainly led to new insights, but the manipulation itself can activate leukocytes and, thereby, influence the pattern of mobilization (Lojek et al., 2002; Triulzi, 2009). Thus, of necessity, the *ex-vivo* labeling method involves extensive perturbation of the cells and the introduction of a relatively small number of labeled cells representing only 1–2% of the total population in the circulation (23).

In contrast to *ex-vivo* labeling methods, in the current study, circulating immune cells were labeled *in vivo* with carboxyfluorescein diacetate succinamidyl ester (CFSE). The injection of CFSE into the circulation is a relatively novel approach that provides for the labeling of the majority of circulating immune cells (Asquith et al., 2006; Becker et al., 2004; Ristevski et al., 2003). CFSE labeling has been used extensively *in vitro* (e.g., Grayson et al., 2003; Wang et al., 2005) and, increasingly, *in vivo* (e.g., Becker et al., 2004; Ristevski et al., 2003) for a variety of cell trafficking studies. CFSE has little or no physiological effect and becomes detectable only after enzymatic activity within the cell has cleaved the molecule and covalently attached it to intracellular macromolecules (Asquith et al., 2006; Becker et al., 2004; Wang et al., 2005). At the concentrations used in the current study, CFSE that is not rapidly taken up by cells is cleared and does not appear to escape the circulation (Asquith et al., 2006; Ristevski et al., 2003). CFSE retained in cells can be detected for several weeks (Becker et al., 2004). Given these properties, we reasoned that CFSE could be used to gauge exercise associated leukocyte trafficking at several intervals over a period of 3–5 days. To our knowledge, this approach has not been used to examine leukocyte trafficking with exercise.

2. Methods

2.1. Animals

All methods and procedures were approved by the University of California Irvine Institutional Animal Care and Use Committee. One hundred Female Sprague–Dawley rats (231 ± 2 g body mass) were randomly assigned to either vehicle (n = 40) or CFSE injection (n = 60) groups. At time 0, rats were injected with either vehicle or CFSE dye. In pilot studies it was established that a relatively rapid decline in circulating CSFE labeled immune cells occurs in the first 120 h (not shown). A temporary nadir in circulating CFSE labeled cells is reached between 120 h and 10 days post-injection with a much slower decline following the 5 day time point. Accordingly, 10 rats from each group were sacrificed at 4, 24 and 120 h after the CFSE injection. Two additional groups of 10 CFSE injected rats were exercised prior to being killed at 24 and 120 h post-injection.

2.2. CFSE preparation and injection

One mg CFSE (Invitrogen) was dissolved in 240 μl DMSO (dimethylsulfoxide) with 10 μl Heparin. The injection dosage was 0.8 μl/g body weight (23). Animals were gently restrained and CFSE (~200 μl bolus) was injected via the tail vein over a 5 min period. To adapt the animals to the injection procedure, they were handled and restrained for 5 min on three separate occasions during the week prior to injections.

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Previous *in vivo* studies with this dye all indicate that little, if any of CFSE following an intravenous injection escapes the circulation and labels cells in extravascular sites. Becker et al. (2004) noted that esterases within the circulation cleave the injected molecules within seconds and the free unconjugated fluorescent dye is rapidly excreted in urine. Asquith et al. (2006) specifically examined the exchange of CFSE labeled lymphocytes between the circulation and lymph nodes. During the time that the majority of lymphocytes were labeled with the injected dye (i.e., within minutes), Asquith and coworkers found no labeled lymphocytes in lymph nodes further indicating that the dye is not labeling extravascular cells. Finally, Ristevski et al. (2003) compared the exchange kinetics of leukocytes labeled intravascularly (*in vivo*) with those labeled *ex vivo* then reintroduced into the circulation. From these comparisons, Ristevski and coworkers reached the conclusion that, “CFSE is not capable of leaving the blood compartment in sufficient quantity to stain lymph node cells.”

2.3. Exercise protocol

Twenty of the rats assigned to the CFSE group were randomly selected for the two exercise subgroups. Exercise consisted of 45 min of treadmill running, ramped to 1 mph for the final 30 min. This final speed would represent ~95% maximum heart rate for rats of this size (Adams et al., 1995). Ten and 5 days prior to CFSE injection, both control and exercised rats experienced five brief bouts (less than 10 min each) of treadmill running to familiarize them with the procedure.

2.4. Tissue collection and analysis

The rats were sacrificed immediately following the exercise challenge using Pentosol euthanasia solution. After the induction of deep anesthesia, but prior to the cessation of breathing, blood was collected from the left ventricle via the diaphragm using a heparinized syringe. The medial gastrocnemius (MG) muscle, spleen and left lobe of the lung were dissected free of connective tissue and weighed.

2.5. Collection of immune cells from tissue

Spleen, lung and muscle tissue single cell suspensions were prepared as follows: tissues were finely minced in cold PBS and the suspension was then passed through a 500 μM cell strainer and then a 70 μM BD (Becton Dickinson) cell strainer consecutively. The resultant suspension was then centrifuged for 5 min at 190*g*. The resultant pellet was resuspended in 1× red blood cell lysing solution (BD) at room temperature and incubated for 3 min. The suspension was washed 3× in BD wash solution (3 ml BD wash solution, resuspend pellet gently and centrifuge for 5 min no brake, repeat 2×). The suspension was then aspirated taking care to avoid any sediment. An aliquot of suspension was removed for cell counting and for flow cytometry. The methods used did not distinguish neutrophils from eosinophils and basophils. However, neutrophils make up the overwhelming majority of the granulocyte pool and therefore any changes seen are assumed to be shifts in the neutrophil population.

For the purposes of this exploratory study, we focused on those tissues whose hemodynamics are known to change substantially with exercise (Maeda et al., 2002). The gastrocnemius was selected as a representative muscle that is particularly active metabolically and hemodynamically with exercise.

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2.6. Flow cytometric data acquisition and analysis

A FACScan (Becton Dickinson, San Jose, CA) was set up for single data acquisition. CFSE was measured in the FL-1 channel (530–615-nm bandpass filter). For each sample, 100,000 events were acquired for analysis. Data acquisition and analysis was performed with CELLQuest software (Becton Dickinson). The analysis gating strategy was as follows: for single-parameter CFSE analysis, all events were plotted in a forward-side scatter plot and gates was set around the high, medium and low forward scatter events constituting the granulocyte, monocyte, and lymphocyte populations, respectively. These gated events were analyzed for CFSE contents and visualized in a histogram.

2.7. Statistical analysis

All values are reported as mean and standard error of the mean (SEM). Between group analysis was conducted using a One-Way ANOVA and SNK post test (PRISM-Graphpad). For all statistical tests the 0.05 level of confidence was accepted for statistical significance. In addition, we modeled the washout of neutrophils, monocytes, and lymphocytes using a simple exponential decay equation: $y = a \cdot e^{-t/\tau}$ where "*a*" is the percent of cells labeled at $t = 0$, "*t*" is time in hours, and τ is the time constant. The $t^{1/2}$ (half-life) is readily calculated from the value of τ. We used standard iterative curve-fitting techniques and statistical analysis to determine the half-life of the circulating neutrophils, lymphocytes, and monocytes. Changes in the proportion of labeled immune cells in the circulation, lung, spleen, and muscle were used to assess the effects of acute exercise on the interchange of leukocytes among the specified compartments.

3. Results

3.1. Circulating neutrophils, lymphocytes, and monocytes—overall kinetics

CFSE injection had no apparent effects on the body or muscle weight of rats over the 5 days of the current study relative to controls (data not shown). The *in vivo* labeling protocol resulted in dye uptake by the majority of circulating immune cells. For example, in blood from animals killed four hours post CFSE injection, the percentage of cells labeled was: monocytes 94 ± 1, lymphocytes 73 ± 2, and neutrophils 90 ± 1. The numbers of labeled cells in the circulation declined over time such that by 5 days post-injection the proportion of labeled cells of each type in the circulation was ~20% (Fig. 1, open circles). The estimated $t^{1/2}$ for circulating neutrophils, lymphocytes, and monocytes was 47 ± 8 h, 56 ± 23 h, and 55 ± 5 h, respectively. For each of the leukocyte subtypes, the exercise bouts prolonged the apparent $t^{1/2}$ presumably via the remobilization of marginated cells.

3.2. Appearance of labeled cells in lung, spleen, and muscle

By 24 h post-injection, the majority of immune cells recovered from lung and spleen tissue were positive for CFSE (Fig. 2). Over time the number of labeled cells declined in the lung and spleen. In contrast, the proportion of CFSE positive cells in muscle increased (Fig. 2).

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3.3. Effect of exercise on target tissue cell labeling

Brief (45 min) exercise challenges at 24 or 120 h post labeling permitted us to gauge the impact of exercise on labeled cell proportions in individual tissues. In the lung, exercise produced a decrease in the number of labeled monocytes, lymphocytes, and neutrophils did not result in significant changes in labeled lymphocytes or neutrophils (Fig. 3), however, the decrease was only significant for the monocytes at both the 24 and 120 h time-points (Fig. 5).

In the spleen (Fig. 4), the proportion of labeled monocytes and lymphocytes was significantly decreased by exercise only at the 120 h time-point. There was no acute effect of exercise on labeled neutrophils in the spleen at either 24 or 120 h.

In the muscle (Fig. 6), there was no acute effect of exercise on neutrophils, lymphocytes, or monocytes at the 24 h time point. However, by 120 h, the proportion of labeled immune cells had increased substantially in the muscle, and significant reductions in monocytes and lymphocytes were observed following exercise at the 120 h time-point. No significant change in muscle neutrophils was observed.

4. Discussion

A major finding of this study is that brief periods of exercise increase the interchange of leukocytes in peripheral compartments (lung, spleen, and muscle) with the central circulation. The lung and spleen exchange more rapidly than the muscle. However, our data indicate for the first time that the interchange of cells between the central circulation and muscle tissue may be quite substantial.

A comparison between our estimates of the circulatory $t^{1/2}$ of neutrophils, lymphocytes, and monocytes and previous estimates provide insights into our current understanding of leukocyte kinetics and the role that exercise might play in these dynamics. As noted, because our first data point was at 4 h, much later than is typically obtained in circulating leukocyte kinetic studies, our study was designed to capture kinetic information on slowly exchanging compartments. Furze and Rankin (2008) recently published a review of neutrophil clearance. In the article’s abstract, the authors state that neutrophils “have a short half-life in the blood of ≊6.5 h.” This value was not obtained from recent experiments, but actually came from classic data published in the early 1960s in which a small number of neutrophils were removed, radioactively labeled, and injected back into the human participants (Athens et al., 1961a,b, 1959; Mauer et al., 1960) (n.b., these studies were performed in prisoners without mention of formal institutional review before such procedures were encoded in law). Mazumder et al. (2010) using unlabeled *t*-helper lymphocyte data from six female participants in a study of the effect of oral contraception on methylprednisone pharmacokinetics (Slayter et al., 1996) calculated a $t^{1/2}$ for lymphocytes as 1.05 h. Finally, studies utilizing radiolabeling of monocytes done in 1970s report the $t^{1/2}$ of monocytes to be 71 h in humans (Whitelaw, 1972) and 42 h in rats (Whitelaw and Batho, 1972).

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The differences between the data presented here in the rat and these previous studies have a number of possible explanations. First, our data suggest that circulating neutrophils, lymphocytes, and monocytes are in contact with both rapid and slowly exchangeable pools. These rapidly exchanging pools include the lung and spleen. Our study strongly suggests that muscle represents a slowly interchanging pool. A possible kinetic model based on our data is shown in Fig. 7 (Landaw et al., 1984; Landaw and DiStefano, 1984). A second consideration is methodological. In previous studies, immune cells were labeled *in vitro* then returned to the circulation. This raises the possibility that the extraction, labeling and re-introduction of the cells may have predisposed them to a much shorter life span. In the current study, the cells were labeled *in vivo* and thus may have been relatively unperturbed.

We did not perform a classic compartmental dynamics study in which we simultaneously sampled the appearance of label in multiple exchanging pools over time in a single animal. Consequently, we could not, for example, determine whether labeled cells identified in muscle tissue were adhering to endothelium within the vascular space or, alternatively, had traversed the vascular wall and were residing within the muscle itself. Obtaining usable samples in a small animal model is challenging and would clearly involve invasive instrumentation and prolonged anesthesia, all factors that could profoundly influence leukocyte kinetics.

There are a number of critical differences between the current work and the earlier studies in humans and rats cited above. First, only a relatively small number of cells, representing 1–2% of the circulating population were labeled in the previous studies. Second the bulk of samples were obtained well before the 4 h point that marked the first sample we obtained in the current study. As a consequence, the calculation of $t^{1/2}$, while experimentally derived and accurate in the earlier studies, would not likely characterize the slowly exchanging pools that we identified in the muscle by examining tissue after 1 and 5 days had elapsed. The relatively stable proportions and high numbers of labeled immune cells in the circulation and tissues demonstrated after these longer time intervals provides an optimal background with which to further assess the impact of physiological challenges on leukocyte trafficking.

Rapid exchange between neutrophils, lymphocytes, and monocytes in the central circulation and the lung is well documented (Doerschuk et al., 1990; Mukae et al., 2001, 2000). The high percentage of labeled cells found in the lung by 24 h along with the subsequent reduction of the proportion of labeled cells by 120 h supports these earlier observations (Fig. 2). We can deduce from our data that with exercise, monocytes may well have left the lung and entered the central circulation since the proportion of labeled monocytes in the lung decreased with exercise at both the 24 and 120 h time-points when simultaneously the proportion of labeled monocytes increased in the central circulation. Lung neutrophils and lymphocytes also decreased with exercise but not significantly, while the proportion of labeled neutrophils in the circulation increased with exercise. Importantly, these observations raise the possibility that, for example, monocytes (and possibly lymphocytes and neutrophils) residing in the lung and affected by conditions such as air pollution (Hollingsworth et al., 2007) or allergens may be rapidly mobilized by exercise back into the central circulation and, ultimately, interact with tissues throughout the body.

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Like the lung, a high proportion of neutrophils, lymphocytes, and monocytes were labeled in the spleen at the 24 h time-point (Fig. 2) and then the proportion of labeled cells decreased. Lymphocytes from the circulation do enter and reside in the spleen, but through a set of mechanisms that differs from lymphocyte adherence in lymph nodes (Grayson et al., 2003). In the case of neutrophils, the classic view is that the shorter-lived neutrophil is destroyed in the liver and spleen (Furze et al., 2008). New data point toward the spleen as a compartment in which a much larger number of true monocytes reside and readily interchange with the circulating blood than had been previously recognized (Swirski et al., 2009). Our data show that on day 5, with exercise, there was a significant reduction in the proportion of labeled lymphocytes and monocytes in the spleen at the same time that the proportion of these labeled cells in the circulation increased. These data suggest that exercise stimulated an egress of these cells from the spleen into the central circulation. In humans, exercise does lead to splenic constriction (Stewart et al., 2003) and might explain, in part, the egress of lymphocytes and monocytes. In contrast, perhaps neutrophils adhere more tightly to the splenic stroma than do monocytes and lymphocytes and, as a consequence, are unaffected by splenic constriction. These observations have potential clinical importance. We speculate, for example, that lymphocytes or monocytes residing in the spleen, activated through the portal circulation to specific antigens in an individual with, for example food allergy, may be subsequently released into the central circulation with exercise. Consistent with this notion, Kruger et al. recently noted in the rat that exercise acutely led to a relocation of lymphocytes from the spleen to the lung (Kruger et al., 2008). This could explain, in part, the well-documented relationship among food, exercise, and anaphylaxis (Du, 2007). Exercise induced anaphylaxis is a rare disorder, but in many cases it appears to be triggered by exercise 1–2 h after an individual with known (or unknown) food allergies ingests a food to which he or she is allergic. The possibility exists, therefore, that exercise redistributes activated immune cells residing in gastrointestinal associated structures (e.g., the spleen) to the central circulation and lung, thereby leading to the disastrous cascade of immune activation associated with anaphylaxis (Robson-Ansley and Toit, 2010).

An intriguing observation of this study was the appearance of labeled neutrophils, lymphocytes, and monocytes in skeletal muscle. Our data suggest that compared with the lung and spleen, this is a relatively slow interchange. Indeed, the proportion of labeled neutrophils, monocytes, and lymphocytes at 24 h was lower in the muscle than in the lung or spleen but increased substantially by day 5 (Fig. 2). We have no explanation for this observation, but it clearly suggest additional unique aspects to immune cell trafficing through muscle, at least in comparison to lung and spleen. The contribution of the slower turnover rate, differences in cell margination, or unique chemotactic properties exhibited by muscle in the current study are not know. It is well established that eccentric exercise that leads to frank muscle damage is associated with accumulation of monocytes and neutrophils at the site of the muscle injury (MacIntyre et al., 2000; Paulsen et al., 2010; Tidball et al., 2010). However, even exercise not typically associated with frank muscle damage leads to accumulation of leukocytes in muscle (Raastad et al., 2003) as does muscle stretch alone (Lockhart and Brooks, 2008). At 5 days, the proportion of labeled lymphocytes and monocytes, but not neutrophils, significantly fell after exercise, indicating a more robust,

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bidirectional exchange of these leukocytes between the muscle and central circulation than had been previously considered.

Only the neutrophils did not demonstrate significant reductions in the proportion of labeling in the lung, spleen, and muscle following exercise. It is possible that neutrophils did return to the central circulation following exercise from the peripheral pools in relatively small numbers such that we could not detect statistical significance, but, collectively, led to the observed significant increases in the proportion of labeled neutrophils in the central circulation. Cell size alone is likely not a factor as monocytes (approximately 14–17 μM) are larger than neutrophils (10–12 μM) or lymphocytes (7–8 μM). In the lung, activation of neutrophils through modulation of adhesion effectors CD11b and L-selectin, occurs as result of homing to the lung itself and not necessarily due to inflammation (Fortunati et al., 2009). This could explain why lung neutrophils in our study seemed not as mobile as the monocytes or lymphocytes. Whether neutrophils become activated in other tissues like the spleen, muscle, or bone marrow (a tissue not examined in this study) as a result of homing itself is not known.

A challenging aspect of this study, indeed, a very knotty methodological problem encountered by all investigators focused on leukocyte trafficking is how to distinguish the subpopulations of cells that "reside" within a particular organ. There are actually two populations of leukocytes that might be "harvested" from a particular tissue: those cells that marginate within an organ's vasculature adhering to the endothelial wall (typically, having activated one or more of the CAM proteins) and those that leave the circulation crossing the vascular barrier into the parenchyma of the organ itself. We did consider perfusing each target tissue following sacrifice with the idea of flushing out leukocytes and identifying labeled cells. We decided against this because in our experience, and in the experience of other researchers (Durowicz and Olszewski, 2003; Menger et al., 2003), such procedures under the unphysiological condition of anesthesia introduce substantial additional sampling error, given the profound alterations in vascular resistance, adhesion molecule production by endothelium, and distribution that occur within individual tissue. Since the animals were passively exsanguinated, any labeled cells that we found within an organ would predominantly be those that were not freely circulating but likely marginated. Thus, any errors that we made by not perfusing or flushing the tissues would tend to underestimate and not overestimate labeled cells within the particular "black box" of a target tissue. Although we did not specifically test for this, residual amount of blood retained in the target organs following removal is likely to be comparable among the various treatment groups and therefore have an equal impact on the *proportions* of labeled cells identified in the lung, spleen and muscle.

It is also important to note that we used only female rats. In our hands, female rats require less prior experience in the treadmill before they will exercise for the prescribed amount of time than do male animals. We recognize that there may be gender differences with regards to cell trafficking. Indeed, Sinha and coworkers observed diminished leukocyte trafficking in female rat aortas compared to male aortas following elastase perfusion (Sinha et al., 2006). Whether exercise alterations in leukocyte trafficking is also influenced by gender is an important topic for future research.

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In summary, these results provide evidence to support the idea that neutrophils, monocytes, and lymphocytes identified in the circulation can exchange bidirectionally with the lung, spleen, and muscle. Exercise tends to favor the movement of lymphocytes, monocytes, and, possibly, neutrophils from these peripheral compartments into the circulation, consistent with the leukocytosis that accompanies even brief exercise. In general, the lung and spleen appear to exchange leukocytes more rapidly with the central circulation than does the muscle. These studies suggest that exercise could hasten the reappearance in the central circulation of leukocytes conditioned by the local environment of the lung, spleen, and muscle providing a potential mechanism through which tissues like muscle could influence immune function systemically. This speculation is consistent with a growing body of data from air pollution to surgery, that *local* triggers can influence *systemic* immune cell function (den Hartigh et al., 2010; Landis, 2009; Menzies et al., 2006; Thomas et al., 2002).

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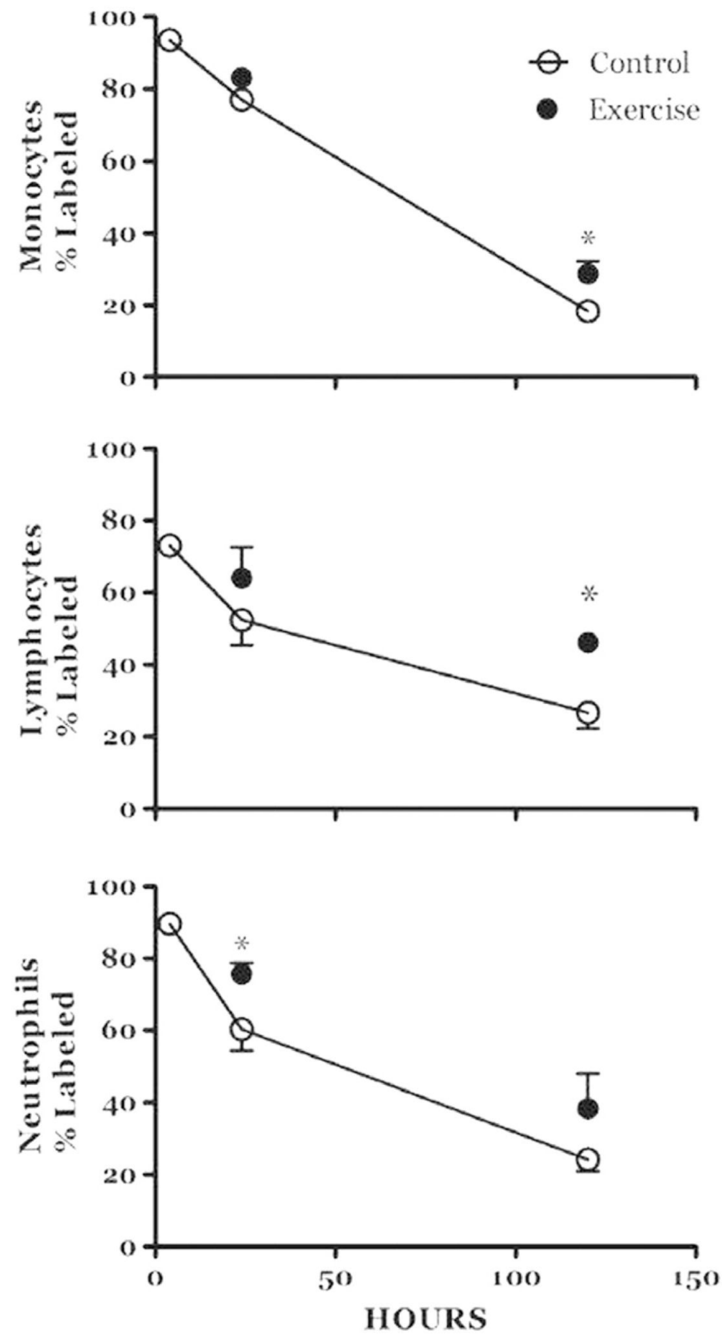

Fig. 1.

The majority of immune cells present in the circulation were labeled with CFSE at 4 h post-injection (open circles). The proportion of CFSE labeled cells declined over time to a nadir at 120 h. Forty-five minutes of running (filled circles) increased the proportions of labeled neutrophils at 24 h and monocytes and lymphocytes at 120 h. *$p < 0.05$ vs. time point control. $N = 10$; monocytes ($F = 284.7$, 3,39 df); lymphocytes ($F = 19.49$, 2,39 df); neutrophils ($F = 62.41$, 3,39 df).

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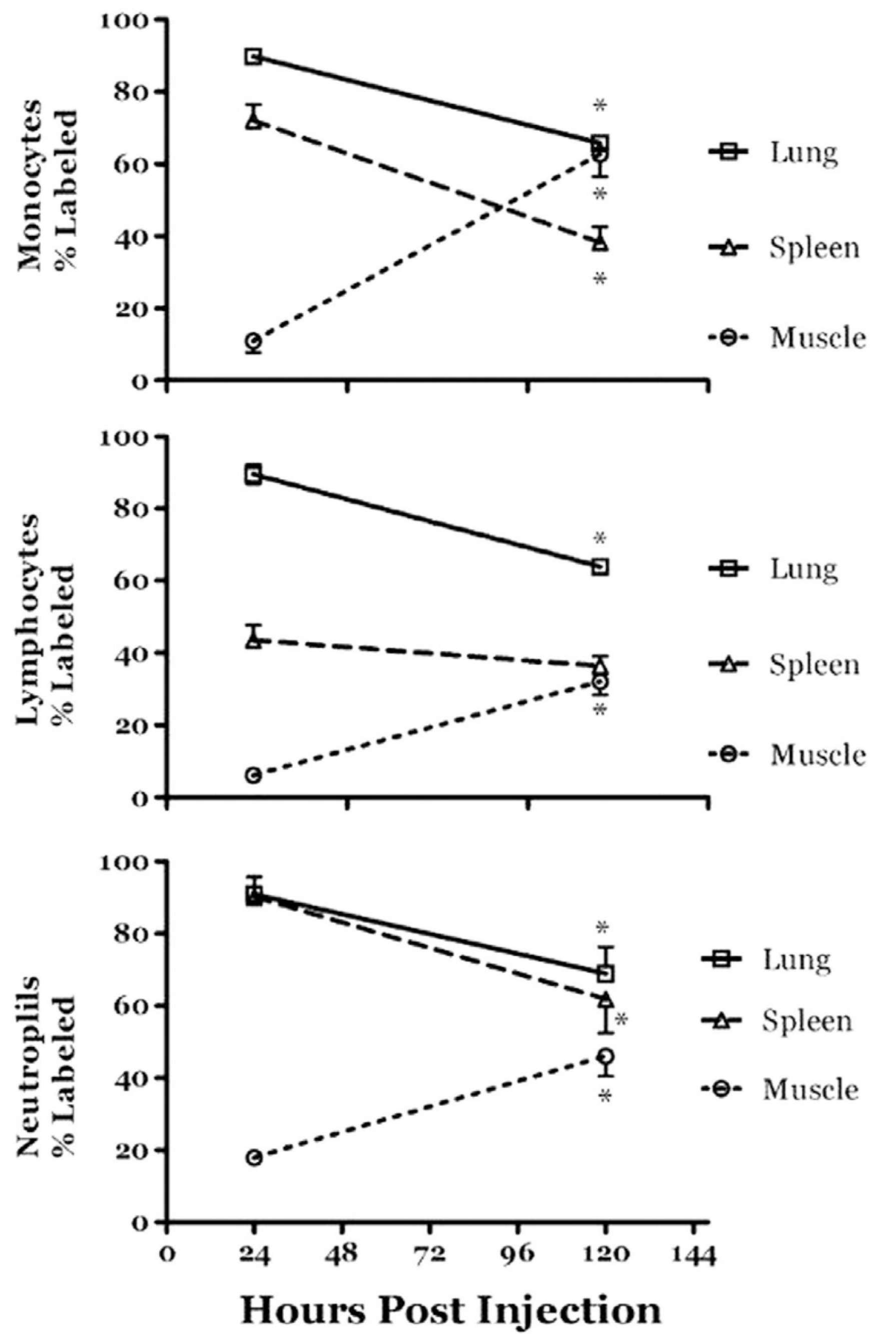

Fig. 2.

The proportion of CFSE labeled immune cells present in lung (open box) and spleen (open triangle) declined and those in skeletal muscle (open circle) increased between 24 and 120 h post-injection. *$p < 0.05$ vs. 24 h value. *F*-ratios reported in Fig. 1 legend.

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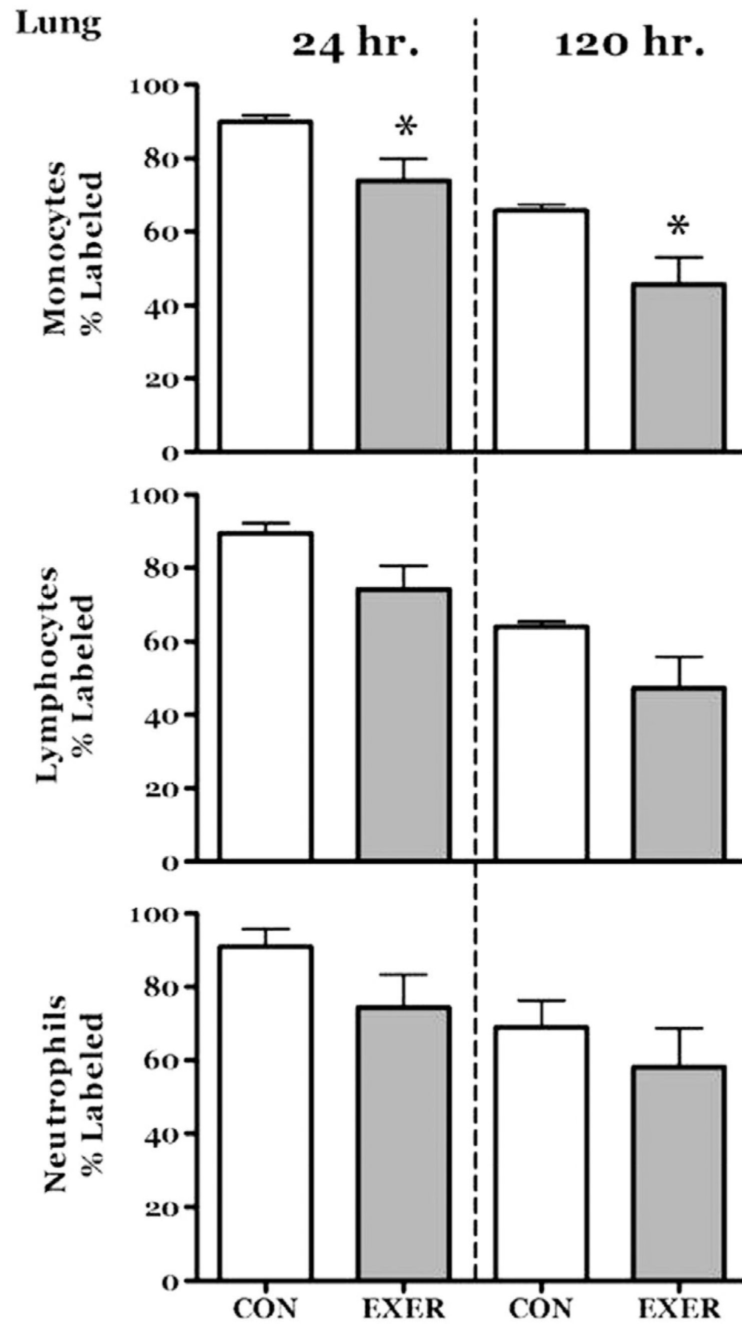

Fig. 3.

At both 24 and 120 h post-injection, 45 min of running (filled bars) resulted in a decrease in the proportion of CFSE labeled monocytes in the lung. *$p < 0.05$ vs. time point control. $N = 10$; ($F = 31.69$, 3,39 df).

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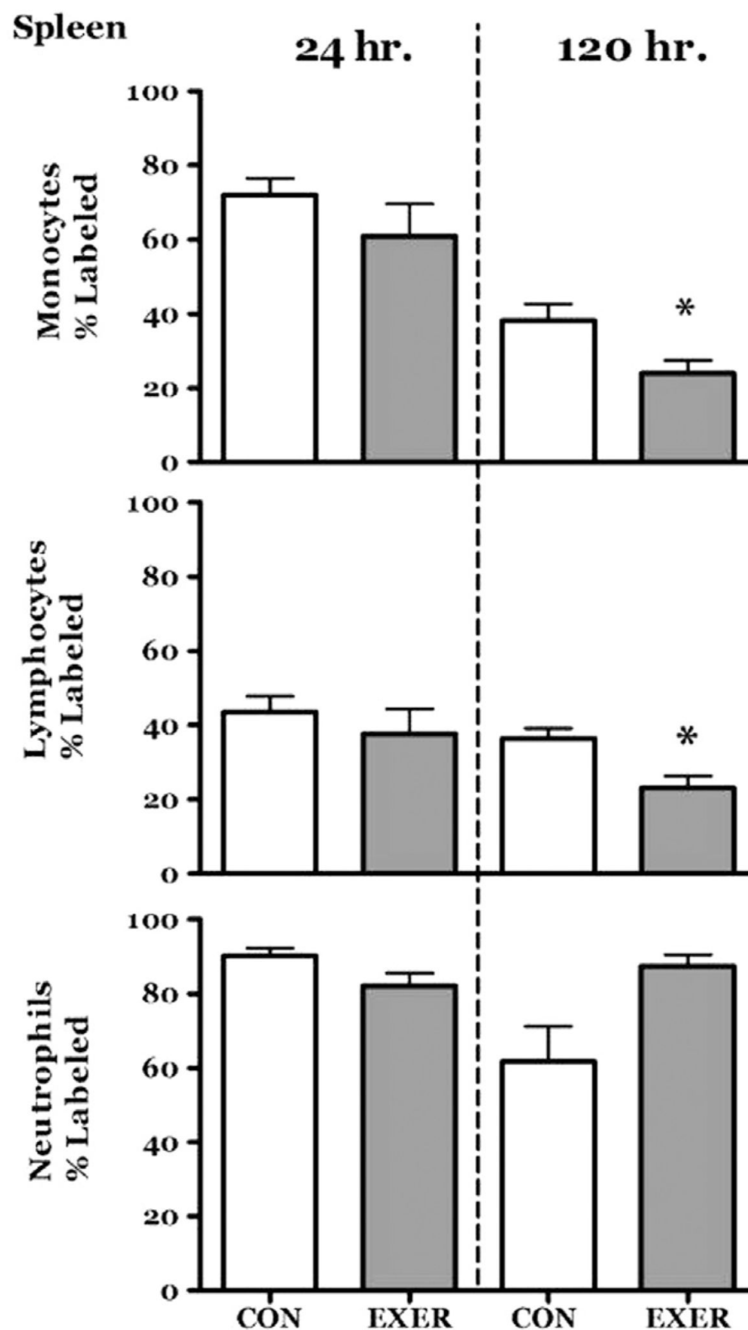

Fig. 4.

At 120 h post-injection, 45 min of running (filled bars) resulted in a decrease in the proportion of CFSE labeled monocytes and lymphocytes in the spleen. $^*p < 0.05$ vs. time point control. $N = 10$; ($F = 26.03$, 3,39 df) and ($F = 4.64$, 3,39 df).

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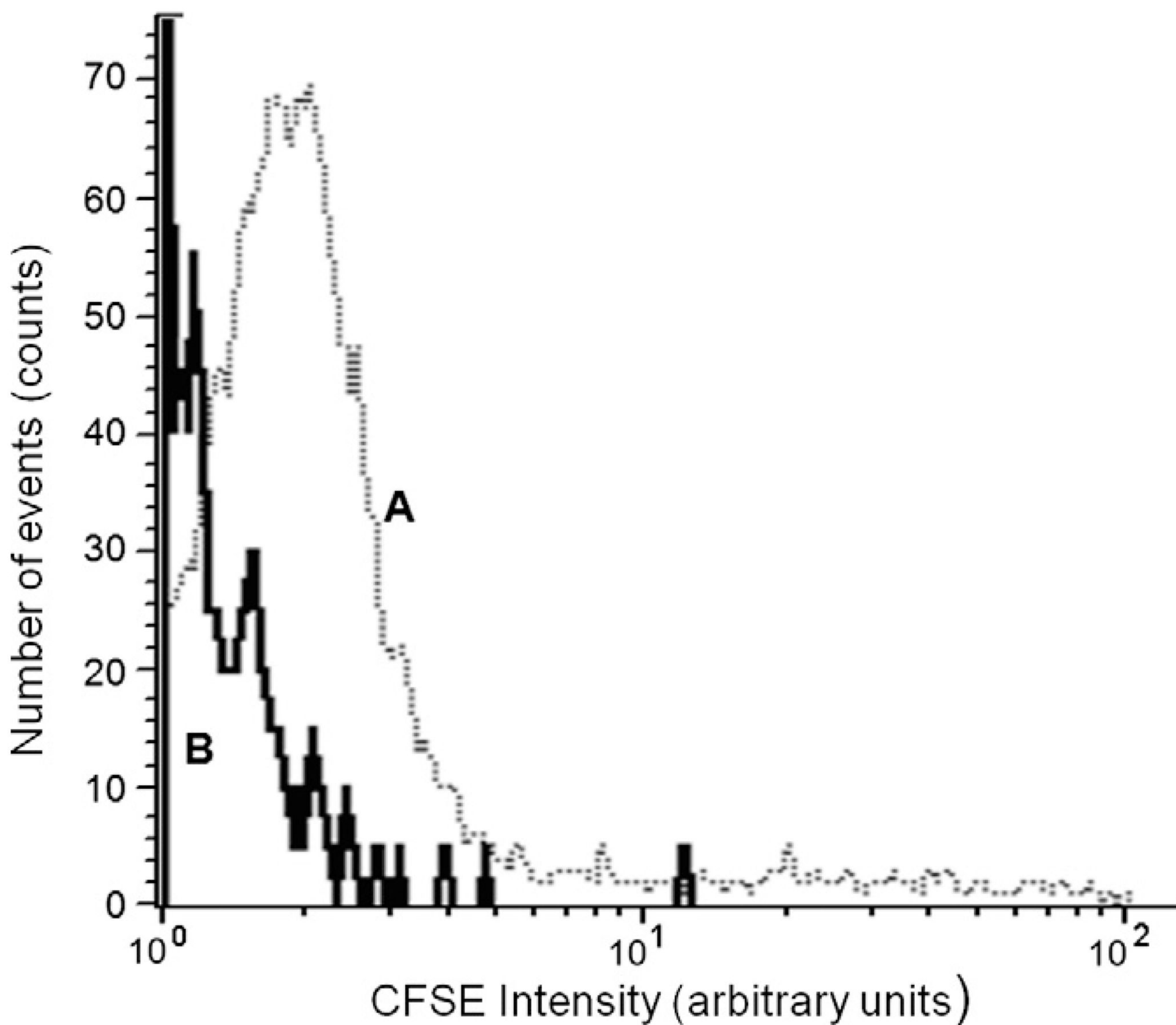

Fig. 5.

Impact of brief exercise on monocytes residing in the muscle tissue. This is a flow cytometer derived example from an unexercised (A) and exercised (B) animal. It illustrates the reduction of labeled monocytes in the gastrocnemius. These data were obtained 120 hours post injection of the CFSE dye.

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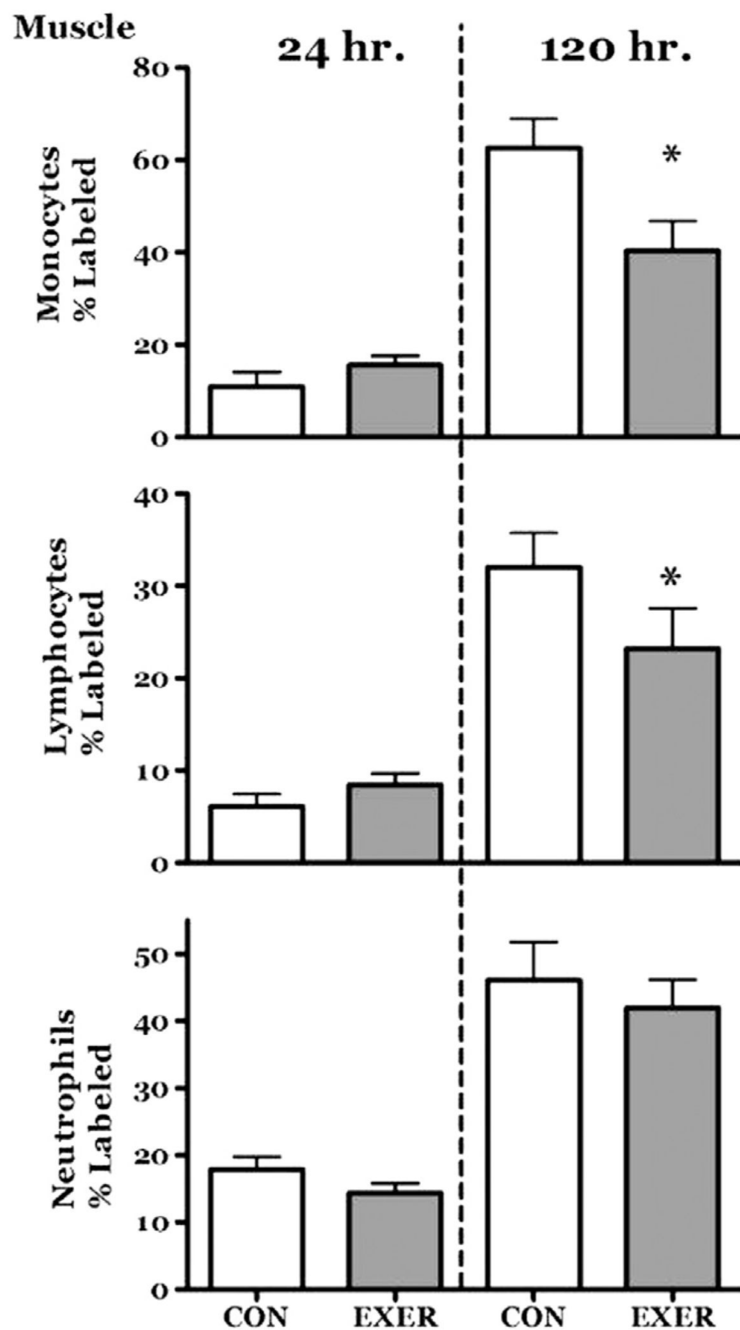

Fig. 6.

At 120 h post-injection, 45 min of running (filled bars) resulted in a decrease in the proportion of CFSE labeled monocytes and lymphocytes in the medial gastrocnemius muscle. $*p < 0.05$ vs. time point control. $N = 10$; ($F = 42.34$, 3,39 df) and ($F = 18.85$, 3,39 df).

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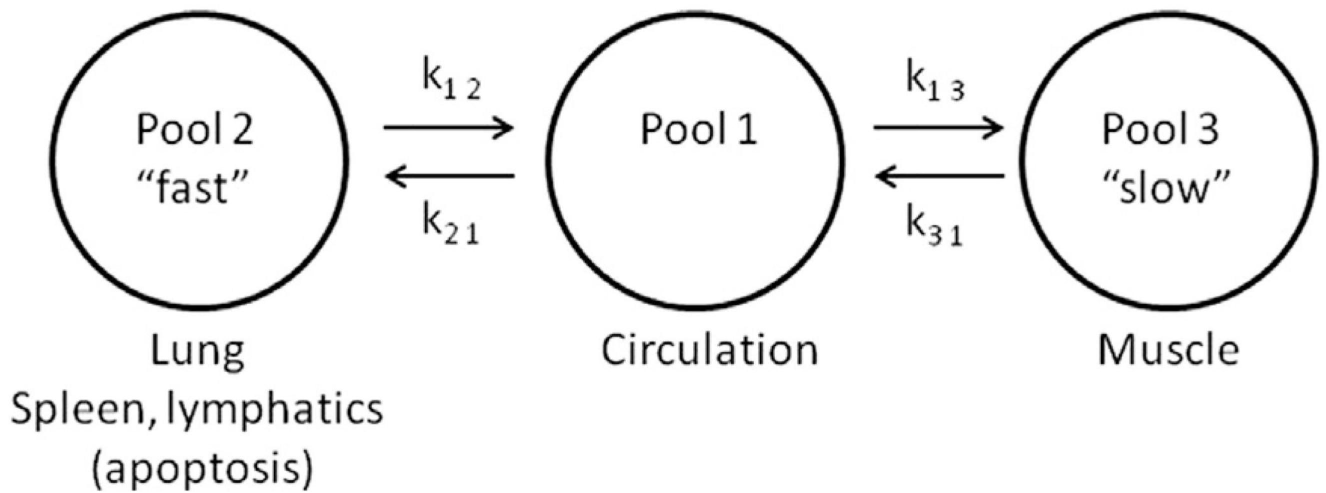

Fig. 7.

A putative, simple kinetic model appropriate to approximate rate constants (“k”) among interchangeable compartments. While our study was not formally designed to calculate pool dynamics, our data suggest that the labeled leukocytes originating in the central circulation rapidly interchange with the lung. The spleen also constitutes a rapidly exchanging pool and may be the site of leukocyte apoptosis and death. Our data suggest that muscle is a more slowly exchanging compartment with possible unique trafficking properties that have yet to be determined.