Impact of training status on LPS-induced acute inflammation in humans

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The overall aim of the present study was to test the hypotheses 1) that training status affects the overall ability to induce an acute inflammatory response systemically, in skeletal muscle and in adipose tissue in human subjects; and 2) that human skeletal muscle contributes to a LPS-induced systemic increase in plasma TNFα and IL-6.

The innate immune system is the first line of defense evolved to protect multicellular organisms from foreign pathogens or toxins and is essential for survival of all species. Immune cells like neutrophils, dendritic cells, monocytes, and fully differentiated tissue resident macrophages are classically seen as the primary cells involved in acute inflammation (1, 22). These sentinel cells sense extracellular and intracellular pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) like the toll-like receptors (TLRs) located on the cell surface. When PAMP binds to its receptor, several intracellular signal cascades are initiated, ultimately leading to transcription and release of proinflammatory cytokines such as tumor necrosis factor (TNF)α and interleukin (IL)–6. TNFα and IL-6 subsequently activate and recruit immune cells to sites of infection, enabling destruction and removal of the pathogens and repair of damaged tissue. However, other cell types like adipocytes and myocytes also express TLRs on the cell surface and have the ability to express and secrete cytokines during various conditions (14, 16, 27). This may indicate that these otherwise metabolic tissues also contribute to the production of inflammatory mediators during acute inflammation.

Epidemiological studies have shown that type 2 diabetes (T2D) patients are more susceptible to various infections than healthy subjects (24, 34, 39). Although T2D is associated with chronic low-grade inflammation, the increased risk of infections in T2D patients may be related to a reduced ability to induce an acute inflammatory response when challenged with an inflammatory stimulus like lipopolysaccharide (LPS) (3, 15). In contrast, exercise training has been suggested to exert anti-inflammatory effects (28, 42) as several studies have reported that a period of exercise training lowers circulating levels of proinflammatory cytokines (20, 25). Moreover, both exercise training and an acute exercise bout have been demonstrated to reduce the expression of TLRs on monocytes, and regular moderate-intensity exercise training has been suggested to protect against respiratory inflammation and infection (26, 30). In addition, a single exercise bout has been reported to reduce an acute LPS-induced plasma TNFα response in humans (37). However, the impact of exercise training on the ability to induce an acute inflammatory response has to our knowledge not been elucidated in humans, and divergent results have been reported in rodents. While 4 wk of exercise training has been reported to attenuate a LPS-induced septic response in rats (10), 3 wk of exercise training has also been demonstrated to enhance LPS-induced cytokine production in isolated murine macrophages (19). In addition, muscle-specific peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α overexpression mice (with skeletal muscles that phenotypically resemble endurance-trained muscles) have been shown to elicit a robust LPS-induced inflammatory response systemically and in skeletal muscle, whereas mice lacking PGC-1α in skeletal muscle have an impaired LPS-induced inflammatory response systemically and in skeletal muscle compared with controls (27). This may suggest that the metabolic profile of skeletal muscle is important for the ability to induce an acute inflammatory response both locally in skeletal muscle and also systemically, but little is known about this in humans.

The overall aim of the present study was to test the hypotheses 1) that training status affects the overall ability to induce an acute inflammatory response systemically, in skeletal muscle and in adipose tissue in human subjects; and 2) that human skeletal muscle contributes to a LPS-induced systemic increase in plasma TNFα and IL-6.
METHODS

Ethical Approval

The study was approved by the Ethics Committee of Copenhagen and Frederiksberg communities (H-1-2012-108) and was conducted in accordance with the guidelines of The Declaration of Helsinki. All subjects were given oral and written information and provided written informed consent prior to the study. Body mass index (BMI), heart rate, mean arterial pressure, body temperature, plasma glucose, and plasma TNFα levels have previously been published for the untrained subjects (5). In addition, PDH-E1α protein content of the UT subjects has previously been used to normalize PDH phosphorylations (5).

Experimental Setup

Subjects and pretesting. The study was a cross-sectional setup; all subjects underwent a medical examination, including a blood test prior to the experimental day, and none had been diagnosed with any overt diseases. Based on self-reported physical activity level and maximum oxygen uptake (VO2max) determined during an incremental bicycle ergometer test, 17 young healthy male subjects were included in the study. Eight subjects with a VO2max above 55 ml O2·min−1·kg body wt−1 and performing exercise training at least three times per week were assigned to a trained group, and nine subjects with a VO2max below 45 ml O2·min−1·kg body wt−1 and not performing regular exercise training were assigned to an untrained group. Body composition was obtained before (Pre) and 60 and 120 min after the LPS injection using the percutaneous needle biopsy technique (4) with suction and under local anesthesia (lidocaine; AstraZeneca, Södertälje, Sweden) from untrained, 0.05.

Analysis

Plasma cytokines. Plasma cytokines were analyzed using an ultra-sensitive MSD multispot 96-well assay system precoated with antibodies (MesoScaleDiscovery, Gaithersburg, MD) according to manufacturer’s protocol.

Plasma free fatty acids (FFAs) were analyzed using a HR series NEFA-HR(2)-kit (WAKO Chemicals, Neuss, Germany) and plasma epinephrine using a 2-CAT plasma ELISAHigh sensitivity-kit (Labor Diagnostika, Nordhorn, Germany) according to manufacturer’s protocols.

Plasma metabolites. Plasma free fatty acids (FFAs) were analyzed using a HR series NEFA-HR(2)-kit (WAKO Chemicals, Neuss, Germany) and plasma epinephrine using a 2-CAT plasma ELISAHigh sensitivity-kit (Labor Diagnostika, Nordhorn, Germany) according to manufacturer’s protocols. The study was a cross-sectional setup; all subjects were assigned to a trained group, and nine subjects with a VO2max determined during an incremental bicycle ergometer test, 17 young healthy male subjects were included in the study. Eight subjects with a VO2max above 55 ml O2·min−1·kg body wt−1 and performing exercise training at least three times per week were assigned to a trained group, and nine subjects with a VO2max below 45 ml O2·min−1·kg body wt−1 and not performing regular exercise training were assigned to an untrained group. Body composition was obtained before (Pre) and 60 and 120 min after the LPS injection using the percutaneous needle biopsy technique (4) with suction and under local anesthesia (lidocaine; AstraZeneca, Södertälje, Sweden) from untrained, 0.05.

Real-time PCR. Total RNA was isolated from 15–20 mg muscle tissue and ~20–40 mg subcutaneous fat by a modified guanidinium thiocyanate-phenol-chloroform extraction method (11) as described previously (31), except that the tissue was homogenized for 3 min at 30 Hz in a TissueLyserII (Qiagen, Hilden, Germany). Total RNA was isolated from ~1·10⁶ PBMCs using Trizol (Life Technologies, Carlsbad, CA). The final RNA pellets were resuspended in DEPC-treated H₂O containing 0.1 mε EDTA. RNA was quantified based on the absorbance at 260 nm (Nanodrop 1000, Thermo Scientific, Rockford, IL). Superscript II RNase H− system and oligo(dT) (Invitrogen, Carlsbad, CA) were used to reverse transcribe the mRNA to cDNA as described previously (31). The amount of single-stranded DNA (ssDNA) was determined in each cDNA sample by use of OliGreen reagent (Molecular Probes, Leiden, The Netherlands) as described previously (21).

Real-time PCR was performed using an ABI 7900 sequence-detection system (Applied Biosystems, Foster City, CA). Primers and TaqMan probes for amplifying gene-specific mRNA fragments were designed using the human-specific database from ensembl.org and Primer Express (Applied Biosystems, Foster City, CA). All TaqMan probes were 5′-FAM and 3′-TAMRA labeled, and primers and TaqMan probes were obtained from TAG Copenhagen (Copenhagen, Denmark) (Table 2). Real-time PCR was performed in triplicates in a total reaction volume of 10 µl using Universal Mastermix (Applied Biosystems, Foster City, CA). Cycle threshold (Ct) was converted to a relative amount by use of a standard curve constructed from a serial dilution of a pooled RT sample analyzed together with the samples. For each sample, target gene mRNA content was normalized to ssDNA content.

Table 1. Basic characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Untrained (n = 9)</th>
<th>Trained (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.4 ± 0.7</td>
<td>24.3 ± 1.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.3 ± 5.0</td>
<td>77.5 ± 2.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.6 ± 1.3</td>
<td>23.4 ± 0.4(#)*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>28.8 ± 2.7</td>
<td>12.4 ± 1.1#*</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>56.7 ± 1.8</td>
<td>64.9 ± 1.9#*</td>
</tr>
<tr>
<td>VO2max, ml O2·min−1·kg−1</td>
<td>40.5 ± 1.5</td>
<td>59.7 ± 1.4#*</td>
</tr>
</tbody>
</table>

All values are given as means ± SE. Part of the measurements from the untrained subjects have previously been published (5). #Significantly different from untrained, P < 0.05. (*)Tendency to be significantly different from untrained, 0.05 ≤ P < 0.1.
**SDS-PAGE and Western blotting.** Protein content and phosphorylation levels were measured in muscle lysates by SDS-PAGE and Western blotting. Equal amounts of total protein were loaded from each sample. Samples from each group were distributed evenly across the gel, and all samples from one subject were loaded on the same gel sample. Commercially available antibodies were used to detect GAPDH (no. 2118), HKII (no. 2867), transcription factor RelA (p65)ser536 (no. 3033), p38 mitogen-activated protein kinases (p38Thr180,Tyr182) (no. 4511), extracellular signal-regulated kinases (ERK; no. 4376)Thr202,Tyr204, inhibitor of HKII PDH-E1alpha, and cyt c than UT (Fig. 1).

**Baseline Skeletal Muscle Metabolic Profile**

The protein content of the metabolic markers of skeletal muscle training status, HKII, PDH-E1alpha, and cyt c was determined in skeletal muscle to verify that the trained subjects (T) not only had a higher cardiovascular fitness, but also a higher metabolic capacity in skeletal muscle than the untrained subjects (UT). T had 1.5- to 2.0-fold higher ($P < 0.05$) basal protein content of HKII, PDH-E1alpha, and cyt c than UT (Fig. 1). The PDH-E1alpha protein content of the UT subjects has previously been used to normalize PDH phosphorylations (5).

**Heart Rate, MAP, and Temperature**

There was no significant difference in the basal heart rate, mean arterial blood pressure, and temperature between UT and T. There was no effect of LPS on MAP or temperature, but there was an overall increase in the heart rate in response to the LPS injection that could not be located (Table 3). The heart rate, MAP, and temperature from the UT subjects have previously been published (5).

**Table 2. Primer and TaqMan probe sequences**

<table>
<thead>
<tr>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>TaqMan Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα 5’-TCCTGCCCAGGGCAGTCAAGAT-3’</td>
<td>5’-AGCTGCCCCTCAGCTTGA-3’</td>
<td>5’-CAAGGCTGTAAGCCCATGTTGATGGAAACCC-3’</td>
</tr>
<tr>
<td>IL-6 5’-ACCCGAAGCTGAAAAGGACA-3’</td>
<td>5’-CTTATTTGGAAGTTCAGCCT-3’</td>
<td>5’-ACAATGCTGAAAAGCAGGAAAGGACTG-3’</td>
</tr>
<tr>
<td>NO2 5’-AGGGGAGATCGTATTCCAGGA-3’</td>
<td>5’-AATAGCCCGACATTCGTTCTG-3’</td>
<td>5’-CGGGGTTGATTATACGAGGTTGAAAC-3’</td>
</tr>
<tr>
<td>TLR4 5’-AGAGGGTGTGCACTGATGACACGTA-3’</td>
<td>5’-GAGCGGAGAGTGTCCACTCTG-3’</td>
<td>5’-TGAGTACAGTGGCTTCCCACCCAGG-3’</td>
</tr>
<tr>
<td>CD68 5’-AGGAGGAAAGGCTGCCAAAGC-3’</td>
<td>5’-TTCAATGCTGTCGATTGATTTGC-3’</td>
<td>5’-TGAGTACAGTGGCTTCCCACCCAGG-3’</td>
</tr>
<tr>
<td>CD206 5’-TTTCTCAGCAAGAAAAACCTCGAACAT-3’</td>
<td>5’-GGTTAATGCTGTTGATITTTGC-3’</td>
<td>5’-GCAAGGCGAGGAAAGGCTGCCAAG-3’</td>
</tr>
</tbody>
</table>

iNOS, inducible nitric oxide synthase; TLR4, toll-like receptor 4; CD68, CD11c, and CD 206, cluster of differentiation 68, -11c, and -206, respectively.

**Plasma Metabolites and Hormones**

Before the LPS injection, the basal plasma glucose level tended to be lower ($P = 0.09$) in T than UT, whereas the basal epinephrine level was 1.8-fold higher ($P < 0.05$) in T than UT. There was no difference in the plasma free fatty acid (FFA) or the plasma cortisol level between UT and T before the LPS injection. The plasma glucose level decreased ($P < 0.05$) 20% 60 min after LPS injection compared with Pre LPS only in UT. FFA and epinephrine increased ($P < 0.05$) 1.5- to 2.4-fold 120 min after the LPS injection compared with Pre in both UT and T with no difference between training status. There was no significant change in the plasma cortisol level in response to LPS injection in either UT or T (Fig. 2).

**Peripheral Blood Mononuclear Cells**

T tended to have lower monocyte ($P = 0.08$) and neutrophil ($P = 0.076$) number in the blood than UT prior to the LPS injection.

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**Fig. 1.** Basal hexokinase (HK) II, pyruvate dehydrogenase-E1alpha (PDH-E1alpha), and cytochrome (Cyt) c protein content in skeletal muscle (SKM) of untrained (UT) and trained (T) subjects. Values are presented as means ± SE in arbitrary units (AU). *Significantly different from UT, $P < 0.05$. 

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**RESULTS**

**Baseline Skeletal Muscle Metabolic Profile**

The protein content of the metabolic markers of skeletal muscle training status, HKII, PDH-E1alpha, and cyt c was determined in skeletal muscle to verify that the trained subjects (T) not only had a higher cardiovascular fitness, but also a higher metabolic capacity in skeletal muscle than the untrained subjects (UT). T had 1.5- to 2.0-fold higher ($P < 0.05$) basal protein content of HKII, PDH-E1alpha, and cyt c than UT (Fig. 1). The PDH-E1alpha protein content of the UT subjects has previously been used to normalize PDH phosphorylations (5).
and 120 min after LPS injection

Table 3. Heart rate, mean arterial pressure, and temperature in untrained and trained subjects before (Pre) and 30, 60, 90, and 120 min after LPS injection

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>UT</td>
<td>T</td>
<td>UT</td>
<td>T</td>
<td>UT</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>62.7 ± 2.9</td>
<td>58.5 ± 3.0</td>
<td>61.2 ± 3.1</td>
<td>57.3 ± 2.6</td>
<td>62.3 ± 2.4</td>
</tr>
<tr>
<td>Temp, °C</td>
<td>37.5 ± 0.1</td>
<td>37.3 ± 0.2</td>
<td>37.1 ± 0.1</td>
<td>37.1 ± 0.2</td>
<td>37.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. HR, heart rate; MAP, mean arterial pressure; Temp, temperature; UT, trained subjects; T, trained subjects.

injection, whereas there was no difference in leukocyte and lymphocyte number between UT and T (Fig. 3). In both UT and T, leukocyte and neutrophil number increased \((P < 0.05)\) ~1.2-fold and ~1.8-fold, respectively, 120 min after the LPS injection compared with Pre, with no difference between training status. The monocyte number decreased \((P < 0.05)\) 60 min after the LPS injection to ~80% of Pre level in both UT and T, but T tended to have a lower \((P = 0.085)\) number of monocytes in the blood than UT at this time point. The monocyte number decreased further 90 and 120 min after the LPS injection, reaching ~25% of the Pre level in both UT and T. The lymphocyte number decreased \((P < 0.05)\) 60 min after the LPS injection to ~90% of Pre only in UT and decreased further in UT to ~65% of Pre at 120 min after the LPS injection. In T, the LPS-induced decrease \((P < 0.05)\) in lymphocyte number was delayed until 90 min after LPS injection but reached the same level as UT at 90 and 120 min after the LPS injection (Fig. 3).

### Plasma Cytokines

The arterial plasma TNFα concentration increased \((P < 0.05)\) ~14-fold in UT and ~12-fold in T 60 min after the LPS injection, where T tended to reach a lower \((P = 0.093)\) level than UT. UT and T reached at 120 min after the LPS injection the same ~19-fold increase in plasma TNFα compared with Pre. The plasma TNFα data from the UT subjects have previously been published (5). The arterial plasma IL-6 concentration increased \((P < 0.05)\) ~17-fold 90 min after the LPS injection relative to Pre only in UT and increased further in UT at 120 min after LPS. The LPS-
induced increase \(P < 0.05\) in plasma IL-6 was “delayed” in T, only reaching statistical significance 120 min after the LPS injection, whereas the absolute level was similar in UT and T (Fig. 4B).

**Isolated Peripheral Blood Mononuclear Cells**

TNF\(\alpha\) mRNA content in PBMC was \(-70\%\) lower \(P < 0.05\) in T than UT. After LPS TNF\(\alpha\) mRNA content in PBMC decreased \(P < 0.05\) 50\% only in UT (Fig. 5A).

There was no difference in basal PBMC IL-6 mRNA between UT and T, and no effect of LPS was observed in the two groups (Fig. 5B).

There was no difference in basal PBMC CD68 and CD206 mRNA content (macrophage markers) between UT and T. After LPS CD68 and CD206 mRNA content in PBMC decreased \(P < 0.05\) 50\% and 20\%, respectively, only in UT (Fig. 5, C and D).

TLR4 and CD11c mRNA as well as mRNA of the inflammatory marker inducible nitric oxide synthase (iNOS) could not be detected in PBMC.

**Adipose Tissue Inflammation**

There was no difference in basal TNF\(\alpha\) mRNA content in adipose tissue between UT and T, and there was an overall increase \(P < 0.05\) in TNF\(\alpha\) mRNA in adipose tissue after the LPS injection in both UT and T (Fig. 6A).

In the basal state, T had \(-70\%\) lower \(P < 0.05\) IL-6 mRNA content in adipose tissue than UT. In response to LPS, IL-6 mRNA increased \(P < 0.05\) \(-5\)-fold at 120 min in T, whereas only a visual increase was present in UT (Fig. 6B).

There was no difference in the basal adipose tissue iNOS mRNA content between UT and T. In UT, adipose tissue iNOS mRNA content increased \(P < 0.05\) \(-3\)-fold 60 min after the LPS injection, whereas there was no effect of LPS on iNOS mRNA content in T (Fig. 6C).

There was no difference in basal adipose tissue TLR4 mRNA content between UT and T. In UT, LPS tended to increase \(P = 0.067\) the TLR4 mRNA content in adipose tissue, whereas there was no effect of LPS on TLR4 mRNA content in T (Fig. 6D).

**Adipose Tissue Macrophage Markers**

In the basal state, T tended to have lower \(P = 0.051\) CD68 mRNA content in adipose tissue than UT, while there was no effect of LPS on CD68 mRNA content in either group (Fig. 7). In accordance, T also tended to have an overall lower \(P = 0.06\) basal CD11c mRNA content in adipose tissue than UT, while there was no effect of LPS on CD11c mRNA content in either group.

There was no difference in basal adipose tissue CD206 mRNA content between UT and T. In UT, LPS tended to...
increase ($P = 0.051$) CD206 mRNA content in adipose tissue.

**Skeletal Muscle Inflammation**

Basal TNFα mRNA content in skeletal muscle tended to be higher ($P = 0.066$) in T than UT. The TNFα mRNA content in skeletal muscle increased ($P < 0.05$) ~2.8- and ~3.5-fold in UT and T, respectively, 120 min after the LPS injection relative to Pre, reaching a ~2.6-fold higher ($P < 0.05$) level in T than UT (Fig. 8A).

There was no difference in basal skeletal muscle IL-6 mRNA content between UT and T. LPS tended to increase ($P = 0.059$) skeletal muscle IL-6 mRNA in UT and increased ($P < 0.05$) skeletal muscle IL-6 mRNA content ~2.4-fold in T, reaching a ~2.1-fold higher ($P < 0.05$) level in T than UT at 120 min after the LPS injection (Fig. 8B).
There was no difference in skeletal muscle iNOS or TLR4 mRNA content between UT and T, and there was no effect of LPS on iNOS and TLR4 mRNA content in skeletal muscle in either group. (Fig. 8, C and D).

**Skeletal Muscle Macrophage Markers**

There was no difference in basal skeletal muscle CD68, CD11c, and CD206 mRNA content between UT and T (Fig. 9).
While LPS increased ($P < 0.05$) skeletal muscle CD68 mRNA content only in T, CD11c mRNA content tended to increase ($P = 0.068$) only in UT after the LPS injection. There was no effect of LPS on skeletal muscle CD206 mRNA content in either group.

Skeletal Muscle Inflammatory Signaling

There was no difference in basal skeletal muscle ERK$^{Thr202,Tyr204}$ phosphorylation between UT and T, and ERK$^{Thr202,Tyr204}$ phosphorylation increased ($P < 0.05$) 2.6- and 3.8-fold in T and UT, respectively, 120 min after the LPS injection relative to Pre, but with no difference between the two groups (Fig. 10). There was no difference in basal skeletal muscle IκB-α and IκB-β protein content or p38$^{Thr180,Tyr182}$, JNK$^{Thr183,Tyr185}$, and p65$^{Ser536}$ phosphorylation between UT and T, and LPS did not affect any of these proteins.
Plasma Cytokine Exchange Across the Leg

T exhibited a net uptake \((P < 0.05)\) of TNF\(_\alpha\) across the leg 60 and 120 min after LPS and UT at 120 min after the LPS injection (Fig. 11A). There was a tendency for an uptake \((P < 0.05)\) of IL-6 in UT 120 min after LPS injection (Fig. 11B), whereas there was no statistically significant IL-6 exchange in T.

**DISCUSSION**

The main findings of the present study are that trained subjects had an enhanced inflammatory response in skeletal muscle and a slightly reduced/delayed systemic and adipose tissue inflammatory response compared with untrained subjects. This overall suggests that training status affects the ability to induce an acute inflammatory response in a tissue-specific manner in human subjects. Moreover, and opposite of the hypothesis, skeletal muscles do not seem to contribute to the systemic increase in plasma TNF\(_\alpha\) and IL-6 in response to LPS in humans.

The novel observation that trained subjects had a delayed LPS-induced increase in plasma TNF\(_\alpha\) and IL-6 relative to untrained subjects may potentially be explained by the seemingly lower monocyte number and lower neutrophil number observed in these subjects. Numerous studies have reported that both epinephrine and cortisol have anti-inflammatory/immunosuppressive effects (7, 23, 32). Hence, epinephrine has previously been reported to inhibit a LPS-induced plasma TNF\(_\alpha\) response in mice (23), and beta-adrenergic receptor blockade has accordingly been shown to attenuate an exercise-induced suppression of LPS-induced TNF\(_\alpha\) production in rats (18). Thus the present observations of higher basal epinephrine level in the trained than the untrained subjects may in part explain the lower basal monocyte/neutrophil number in the untrained than the trained subjects and may have contributed to the delayed LPS-induced increase in plasma TNF\(_\alpha\) and IL-6 in the trained subjects relative to untrained subjects. However, it should also be noted that no difference was observed between trained and untrained subjects in immune cell number or...
plasma TNFα and IL-6 120 min after the LPS injection, highlighting that untrained and trained subjects reached a similar systemic LPS-induced inflammatory response later in the trial.

The finding that neither TNFα nor IL-6 mRNA content increased in isolated PBMCs suggests that the systemic levels of TNFα and IL-6 did not originate from circulating PBMCs. Furthermore, the observation that TNFα, CD68 and CD206 mRNA content in PBMC decreased in the untrained subjects after the LPS injection, which may suggest that PBMCs including macrophages were recruited to the peripheral tissues in response to LPS.

The general LPS-induced increase in adipose tissue inflammatory markers is in accordance with previous studies (27, 33) and underlines the capability of this tissue to express these cytokines. The observations that adipose tissue iNOS and to some extent adipose tissue TLR4 mRNA only increased in the untrained subjects in response to LPS, while IL-6 mRNA only increased significantly in the trained subjects, indicate that the LPS-induced inflammatory response in adipose tissue differed between untrained and trained subjects. This is in part in accordance with a recent mouse study, reporting that 4 wk of exercise training attenuated a CL 316,243-mediated induction of inflammatory genes in epididymal adipose tissue (9). However, it should be noted that epididymal (visceral) and subcutaneous adipose tissue have very distinct inflammatory profiles (36, 40), and Castellani et al. (9) reported that the effects of exercise training were solely present in epididymal and not in subcutaneous adipose tissue, as in the present study. The physiological relevance of these differences remains to be determined. The present finding that untrained subjects had a higher adipose tissue CD68 and CD11c mRNA content than trained subjects suggests that the adipose tissue of untrained subjects was more infiltrated with macrophages than the trained subjects and may in part explain the seemingly more pronounced adipose tissue inflammatory response in the untrained subjects than the trained subjects. The higher CD68 and CD11c mRNA content in adipose tissue from untrained than trained subjects is in accordance with a previous study in rodents reporting that physically inactive diet-induced obese rodents had more macrophages in adipose tissue than nonobese rodents (17) and that exercise training prevented the infiltration of macrophages in adipose tissue. Similarly, obesity has been reported to be associated with macrophage infiltration in adipose tissue in humans (41), which together with the present observations suggests that the overall metabolic status determines the degree of macrophage infiltration in adipose tissue. The LPS-induced increase in TNFα and IL-6 mRNA in skeletal muscle in the present study is in accordance with previous studies showing that myocytes in culture (14, 27) and skeletal muscle in vivo (2, 14, 27) produce pro-inflammatory cytokines when exposed to LPS. The observation that trained subjects had a more robust LPS-induced increase in TNFα and IL-6 mRNA content in skeletal muscle than untrained subjects has to our knowledge not previously been shown. However, mice with a high metabolic and oxidative capacity have previously been reported to have a more pronounced LPS-induced inflammatory response than mice with a reduced metabolic and oxidative capacity (27). Similarly, the higher skeletal muscle content of HKII, PDH-E1α, and cyt c in the trained subjects than the untrained in the present study overall supports that skeletal muscle metabolic/oxidative capacity may be important for the ability to induce acute inflammation in skeletal muscle. The underlying mechanism behind the different LPS-induced inflammatory responses in skeletal muscle in untrained and trained subjects is, however, difficult to explain. The present observations that there were no differences in the inflammatory signaling pathways or in basal TLR4 and CD68 mRNA content in skeletal muscle between untrained and trained subjects overall indicate that neither receptor signaling nor resident macrophages within skeletal muscle were responsible for the observed differences. It should be noted that while there was an
overall LPS-induced increase in CD68 mRNA content in skeletal muscle only in the trained subjects, CD11c mRNA content tended to increase in response to the LPS injection only in the untrained subjects. However, elucidation of the mechanism behind the different LPS-induced responses in these macrophage markers in untrained and trained skeletal muscle may require for example FACS analyses in future studies.

The novel finding that both untrained and trained subjects had a positive TNFα and IL-6 A-V difference across the leg, despite the observed LPS-induced increase in TNFα and IL-6 mRNA in skeletal muscle indicates that TNFα and IL-6 were taken up rather than released from the muscle after the LPS injection, which is opposite of our hypothesis. This may overall suggest that skeletal muscle does not contribute to the systemic increases in TNFα and IL-6 during LPS-induced acute inflammation. Furthermore, the apparent uptake of TNFα and IL-6 into skeletal muscle may be due to a general liquid shear and accumulation of fluids in the peripheral tissues as a consequence of the inflammatory state. However, a potential secretion of TNFα and IL-6 with concomitant local (auto- or paracrine) effects as previously suggested (29, 38) cannot be excluded and remains to be determined.

The observations that the LPS-induced adipose tissue and systemic responses were reduced and the skeletal muscle response enhanced in trained subjects compared with untrained subjects suggest that different cell types respond differently to LPS potentially depending on whole body metabolic status. This is in accordance with a recent study performed in horses reporting that training status differentially affected the innate immune response in blood monocytes and pulmonary alveolar macrophages (13). While the present study examined early and peak LPS-induced inflammatory responses, a previous study performed in elderly subjects showed a prolonged inflammatory activity during pneumococcal infections (14). The novel finding that both untrained and trained subjects show prolonged in vivo inflammatory activity during pneumococcal infections (14, 37) is in accordance with a recent study performed in horses.

In conclusion, the present study shows that training status has a tissue-specific impact on LPS-induced acute inflammation and that skeletal muscles do not seem to contribute to the LPS-induced increases in systemic TNFα and IL-6.

REFERENCES
12. Frelstedt L, Waldschmidt I, Gosset P, Desmet C, Pirottin D, Bureau Bergstrom J. Priming of macrophage markers in untrained and trained skeletal muscle may be due to a general liquid shear and accumulation of fluids in the peripheral tissues as a consequence of the inflammatory state. However, a potential secretion of TNFα and IL-6 with concomitant local (auto- or paracrine) effects as previously suggested (29, 38) cannot be excluded and remains to be determined. The novel finding that both untrained and trained subjects had a positive TNFα and IL-6 A-V difference across the leg, despite the observed LPS-induced increase in TNFα and IL-6 mRNA in skeletal muscle indicates that TNFα and IL-6 were taken up rather than released from the muscle after the LPS injection, which is opposite of our hypothesis. This may overall suggest that skeletal muscle does not contribute to the systemic increases in TNFα and IL-6 during LPS-induced acute inflammation. Furthermore, the apparent uptake of TNFα and IL-6 into skeletal muscle may be due to a general liquid shear and accumulation of fluids in the peripheral tissues as a consequence of the inflammatory state. However, a potential secretion of TNFα and IL-6 with concomitant local (auto- or paracrine) effects as previously suggested (29, 38) cannot be excluded and remains to be determined. The observations that the LPS-induced adipose tissue and systemic responses were reduced and the skeletal muscle response enhanced in trained subjects compared with untrained subjects suggest that different cell types respond differently to LPS potentially depending on whole body metabolic status. This is in accordance with a recent study performed in horses reporting that training status differentially affected the innate immune response in blood monocytes and pulmonary alveolar macrophages (13). While the present study examined early and peak LPS-induced inflammatory responses, a previous study performed in elderly subjects showed a prolonged inflammatory response in elderly relative to young when challenged with a pneumococcal infection (8). It may therefore be speculated that additional effects of training status exist in the resolution of the inflammatory response.

In conclusion, the present study shows that training status has a tissue-specific impact on LPS-induced acute inflammation and that skeletal muscles do not seem to contribute to the LPS-induced increases in systemic TNFα and IL-6.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


