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

J. Olesen,1 R. S. Biensø,1 S. Meinertz,1 L. van Hauen,1 S. M. Rasmussen,1 L. Gliemann,2 P. Plomgaard,1,3 and H. Pilegaard1

1Centre of Inflammation and Metabolism and Centre for Physical Activity Research, Department of Biology, University of Copenhagen, Copenhagen, Denmark; 2Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark; and 3Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark

Submitted 12 August 2014; accepted in final form 28 December 2014

Olesen J, Biensø RS, Meinertz S, van Hauen L, Rasmussen SM, Gliemann L, Plomgaard P, Pilegaard H. Impact of training status on LPS-induced acute inflammation in humans. J Appl Physiol 118: 818–829, 2015. First published December 30, 2014; doi:10.1152/japplphysiol.00725.2014.—The aim of the present study was to examine the impact of training status on the ability to induce a lipopolysaccharide (LPS)-induced inflammatory response systemically as well as in skeletal muscle (SkM) and adipose tissue (AT) in human subjects. Seventeen young (23.8 ± 2.5 yr of age) healthy male subjects were included in the study with eight subjects assigned to a trained (T) group and nine subjects assigned to an untrained (UT) group. On the experimental day, catheters were inserted in the femoral artery and vein of one leg for blood sampling and a bolus of 0.3 ng LPS/kg body wt was injected into an antecubital vein in the forearm. Femoral arterial blood flow was measured by ultrasound Doppler, and arterial and venous blood samples were drawn before (Pre) LPS injection and 30, 60, 90, and 120 min after the LPS injection. Vastus lateralis muscle and abdominal subcutaneous AT biopsies were obtained Pre and 60 and 120 min after the LPS injection. LPS increased the systemic plasma TNFα and IL-6 level as well as the TNFα and IL-6 mRNA content in SkM and AT of both UT and T. However, whereas the LPS-induced inflammatory response in SkM was enhanced in T subjects relative to UT, the inflammatory response systemically and in AT was somewhat delayed in T subjects relative to UT. The present findings highlight that training status affects the ability to induce a LPS-induced acute inflammatory response in a tissue-specific manner.

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.

Address for reprint requests and other correspondence: H. Pilegaard, August Krogh Bldg., Universitetsparken 13, Univ. of Copenhagen, Copenhagen 2100 KBH Ø, Denmark (e-mail: hpilegaard@bio.ku.dk).
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 ($V\dot{O}_{2\max}$) determined during an incremental bicycle ergometer test, 17 young healthy male subjects were included in the study. Eight subjects with a $V\dot{O}_{2\max}$ above 55 ml O$_2$·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 $V\dot{O}_{2\max}$ below 45 ml O$_2$·min$^{-1}$·kg body wt$^{-1}$ and not performing regular exercise training were assigned to an untrained group. Body composition was measured by DXA scanning (see Table 1 for subject characteristics).

Experimental day. The subjects were instructed to refrain from moderate- to high-intensity exercise 24 h prior to the experiment and to consume a low-fat meal 1 h prior to arrival. The participants arrived to the laboratory with a minimum of physical activity and were placed in the supine position in a hospital bed. A catheter was inserted in the femoral artery and vein of one leg for blood sampling. Two pilot experiments were conducted using 0.5 and 0.8 ng LPS/kg body wt based on previous observations (12); however, these doses resulted in a dramatic drop in blood pressure during the invasive procedures in wt based on previous observations (12); however, these doses resulted in a dramatic drop in blood pressure during the invasive procedures in the present setup. Instead, the final experiments were conducted with a bolus of 0.3 ng $E$. coli LPS/kg body wt (diluted in 10-ml sterile saline) injected into an antecubital vein in the forearm. Vastus lateralis muscle and abdominal subcutaneous adipose tissue biopsies were obtained before (Pre) and 60 and 120 min after the LPS injection under local anesthesia (lidocaine; AstraZeneca, Södertälje, Sweden) using the percutaneous needle biopsy technique (4) with suction and venous blood samples were drawn from the catheters, and femoral arterial blood flow was measured by ultrasound Doppler (Logic E9, GE Healthcare, Pittsburgh, PA) Pre and 30, 60, 90, and 120 min after the LPS injection. A portion of the arterial blood was collected in heparin-coated tubes, and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation, as previously described (35), and stored at −80°C for later analyses. Body temperature, heart rate, and blood pressure were measured every 15 min throughout the experiment, and blood pressure measurements continued ~2–3 h after cessation of the protocol.

Analyses

Plasma cytokines. Plasma cytokines were analyzed using an ultra-sensitive MSD multiplex 96-well assay precoated with antibodies (MesoScaleDiscovery, Gaithersburg, MD) according to manufacturer’s protocol. The MSD plates were measured on a MSD Sector Imager 2400 plate reader. Raw data were measured as electrochemiluminescence signal (light) detected by photodetectors and analyzed using the Discovery Workbench 3.0 software (MSD). A standard curve was generated for each analyte and used to determine the concentration of analytes in each sample.

RNA isolation, RT, and real-time PCR. Total RNA was isolated from 15–20 mg muscle tissue and ~20–40 mg subcutaneous fat by a modified guanidinium thiocyanate-pheno1-chloroform extraction method (11) as described previously (31), except that the tissue was homogenized for 3 min at 30 Hz in a TissueLyser II (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 H$_2$O containing 0.1 mE DNA. 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.

Lyase generation and protein determination. Freeze-dried muscle biopsies were dissected free of visible fat, blood, and connective tissue under a stereomicroscope in a temperature (~18°C)-controlled and humidity (~30%)-controlled room. Muscle lysate was produced from ~5–10 mg dry wt muscle as previously described (6), except that the tissue was homogenized for 2 min at 30 Hz in a TissueLyser (TissueLyser II; Qiagen, Hilden, Germany). Homogenates were centrifuged for 20 min at 16,000 g, 4°C, and lysates (supernatant) were collected. Protein content in lysates was measured by the bicinchoninic acid (BCA) method (Thermo Scientific, Rockford, IL).

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>$V\dot{O}_{2\max}$, ml O$_2$·min$^{-1}$·kg body wt$^{-1}$</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.
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’-TCTGCCCCAGGCTAGTCA-3’</td>
<td>5’-AGCTGGCTGCTGAGTTGA-3’</td>
<td>5’-CAAGGCTGTAGGCGATGTAGA-3’</td>
</tr>
<tr>
<td>IL-6 5’-CTGGAGCTGGAAGGTTCA-3’</td>
<td>5’-CTTTCTCTCTCAGTTGCT-3’</td>
<td>5’-AGATGGCTGTTATATGCGGAT-3’</td>
</tr>
<tr>
<td>iNOS 5’-ACCTGGACGCCAGGCTGAAT-3’</td>
<td>5’-ATAGGGGATGACTTCCAAGA-3’</td>
<td>5’-CAGGGCTGTTATATGCGGAT-3’</td>
</tr>
<tr>
<td>TLR4 5’-AGGGCTGGAGTTGCT-3’</td>
<td>5’-GAGCCCGAGATGGTCAAGGT-3’</td>
<td>5’-TGGAGTACATATGCGGAT-3’</td>
</tr>
<tr>
<td>CD68 5’-AGAGGGTGGTATCAGTACACGT-3’</td>
<td>5’-GGATGGCTGAGGAGAATCT-3’</td>
<td>5’-TGGAGTACATATGCGGAT-3’</td>
</tr>
<tr>
<td>CD11c 5’-AGGGCGAGGGCGTGGAGCG-3’</td>
<td>5’-TTGTAATGCTGCTGATGTATAGG-3’</td>
<td>5’-TGGAGTACATATGCGGAT-3’</td>
</tr>
<tr>
<td>CD206 5’-TTGGGCTGGGATGACTTTCCAAGA-3’</td>
<td>5’-GTTGGTCTGGTCAATGATGTA-3’</td>
<td>5’-TGGAGTACATATGCGGAT-3’</td>
</tr>
</tbody>
</table>

*InOS, inducible nitric oxide synthase; TLR4, toll-like receptor 4; CD68, CD11c, and CD206, cluster of differentiation 68, -11c, and -206, respectively.

**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. Commercially available antibodies were used to detect GAPDH (no. 2118), HKII (no. 2867), transcription factor RelA (p65) (no. 3033), p38 mitogen-activated protein kinases (p38) (Th180, Tyr182) (no. 4511), extracellular signal-regulated kinases (ERK; no. 4376), and JNK (no. 9251) (all from Cell Signalling, Danvers, MA); cyt c (no. 556433, BP Pharmingen, San Diego, CA); and PDH-E1α (a kind gift from Graham Hardie, Dundee). Band intensity was quantified using ImageQuant LAS 4000 (GE Healthcare, Gaithersburg, MD) and ImageQuant TL v8.1.0.0 software. Protein content is expressed as arbitrary units relative to a standard loaded on each site of each gel.

**Statistical Analyses**

Values above or below means ± 2 SD were considered outliers and excluded from the dataset. Two-way repeated-measures ANOVA was applied to test the effect of LPS treatment and the effect of training state (trained vs. untrained). One-way repeated-measures ANOVA was applied to test the effect of LPS treatment and the effect of training state (trained vs. untrained). There was no difference in the plasma free fatty acid (FFA) or the plasma cortisol level between UT and T before the LPS injection. Plasma glucose level decreased (P < 0.05) 20% 60 min after LPS injection compared with Pre in both UT and T with no difference between training status. There was no significant change in the plasma corticosterol 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 that could not be located (Table 3). The heart rate, MAP, and temperature from the UT subjects have previously been published (5).

**Results**

**Baseline Skeletal Muscle Metabolic Profile**

The protein content of the metabolic markers of skeletal muscle training status, HKII, PDH-E1α, 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-E1α, and cyt c than UT (Fig. 1). The PDH-E1α 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. There was no significant difference in the basal heart rate, MAP, and temperature from the UT subjects has previously been published (5).
and 120 min after LPS injection

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 3).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>UT</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>62.7 ± 2.9</td>
<td>58.5 ± 3.0</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>87.6 ± 2.6</td>
<td>83.9 ± 2.2</td>
</tr>
<tr>
<td>Temp, °C</td>
<td>37.5 ± 0.1</td>
<td>37.3 ± 0.2</td>
</tr>
<tr>
<td>30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>62.1 ± 3.1</td>
<td>57.3 ± 2.6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>84.8 ± 3.4</td>
<td>83.1 ± 3.3</td>
</tr>
<tr>
<td>Temp, °C</td>
<td>37.1 ± 0.1</td>
<td>37.1 ± 0.2</td>
</tr>
<tr>
<td>60 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>62.3 ± 2.4</td>
<td>55.2 ± 1.0</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86.7 ± 3.2</td>
<td>83.1 ± 3.1</td>
</tr>
<tr>
<td>Temp, °C</td>
<td>37.1 ± 0.1</td>
<td>37.4 ± 0.2</td>
</tr>
<tr>
<td>90 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>64.7 ± 2.3</td>
<td>61.1 ± 1.8</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86.8 ± 2.7</td>
<td>82.5 ± 2.3</td>
</tr>
<tr>
<td>Temp, °C</td>
<td>37.2 ± 0.1</td>
<td>37.3 ± 0.2</td>
</tr>
<tr>
<td>120 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>67.2 ± 3.1</td>
<td>63.2 ± 2.3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>89.6 ± 3.1</td>
<td>86.4 ± 1.9</td>
</tr>
<tr>
<td>Temp, °C</td>
<td>37.2 ± 0.1</td>
<td>37.6 ± 0.2</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) (Fig. 4A).

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.

![Fig. 2. Arterial plasma glucose (A), free fatty acids (B), cortisol (C), and epinephrine (D) in UT and T subjects before (Pre) and 30, 60, 90 and 120 min after LPS injection (A and C) and Pre, 60, and 120 min after LPS injection (B and D). The plasma glucose level at Pre and at 120 min from UT has previously been published (5). Values are presented as means ± SE. #Significantly different from UT, P < 0.05. †Tendency to be significantly different from UT, 0.05 ≤ P < 0.1. *Significantly different from Pre within UT, P < 0.05. †Significantly different from Pre within T subjects, P < 0.05.](http://jap.physiology.org/DownloadedFrom/10.1152/japplphysiol.00725.2014)
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**

TNFα mRNA content in PBMC was ~70% lower ($P < 0.05$) in T than UT. After LPS TNFα 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 TNFα mRNA content in adipose tissue between UT and T, and there was an overall increase ($P < 0.05$) in TNFα 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 TNF\(\alpha\) mRNA content in skeletal muscle tended to be higher \((P = 0.066)\) in T than UT. The TNF\(\alpha\) mRNA content in skeletal muscle increased \((P < 0.05)\) \(\sim 2.8\)- and \(\sim 3.5\)-fold in UT and T, respectively, 120 min after the LPS injection relative to Pre, reaching a \(\sim 2.6\)-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 \(\sim 2.4\)-fold in T, reaching a \(\sim 2.1\)-fold higher \((P < 0.05)\) level in T than UT at 120 min after the LPS injection (Fig. 8B).

Fig. 4. Plasma TNF\(\alpha\) (A) and plasma IL-6 (B) in untrained (UT) and trained (T) subjects before (Pre) and 30, 60, 90, and 120 min after LPS injection. Values are presented as means \(\pm SE\). (#)Tendency to be significantly different from UT, \(0.05 \leq P < 0.1\). *Significantly different from Pre within UT, \(P < 0.05\). †Significantly different from Pre within T subjects, \(P < 0.05\).

Fig. 5. TNF\(\alpha\) (A), IL-6 (B), cluster of differentiation (CD) 68 (C) and CD206 (D) mRNA content in isolated peripheral blood mononuclear cells (PBMC) from untrained (UT) and trained (T) subjects before (Pre) and 120 min after LPS injection. Values are presented as means \(\pm SE\). #Significantly different from UT, \(P < 0.05\). *Significantly different from Pre within UT, \(P < 0.05\).
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 \( (P < 0.052) \) 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 CL316,243-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 reporting that training status differentially affected the innate system was reduced and the skeletal muscle response enhanced in trained subjects compared with 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.

REFERENCES
20. Kohut ML, McCann DA, Russell DW, Konopka DN, Cunnick JE, Franke WD, Castillo MC, Reighard AE, Vanderah T. Aerobic exercise, but not flexibility/resistance exercise, reduces serum IL-1β, CRP, and