Exercise-associated generation of PPARγ ligands activates PPARγ signaling events and upregulates genes related to lipid metabolism

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Thomas AW, Davies NA, Moir H, Watkeys L, Ruffino JS, Isa SA, Butcher LR, Hughes MG, Morris K, Webb R. Exercise-associated generation of PPARγ ligands activates PPARγ signaling events and upregulates genes related to lipid metabolism. J Appl Physiol 112: 806–815, 2012. First published December 15, 2011; doi:10.1152/japplphysiol.00864.2011.—The aim of the present study was to test the hypotheses that exercise is associated with generation of peroxisome proliferator-activated receptor-γ (PPARγ) ligands in the plasma and that this may activate PPARγ signaling within circulating monocytes, thus providing a mechanism to underpin the exercise-induced antiatherogenic benefits observed in previous studies. A cohort of healthy individuals undertook an 8-wk exercise-training program; samples were obtained before (Pre) and after (Post) standardized submaximal exercise bouts (45 min of cycling at 70% of maximal O2 uptake, determined at baseline) at weeks 0, 4, and 8. Addition of plasma samples to PPARγ response element (PPRE)-luciferase reporter gene assays showed increased PPARγ activity following standardized exercise bouts (Post/Pre = 1.23 ± 0.10 at week 0, P < 0.05), suggesting that PPARγ ligands were generated during exercise. However, increases in PPARγ/PPRE-luciferase activity in response to the same standardized exercise bout were blunted during the training program (Post/Pre = 1.18 ± 0.14 and 1.10 ± 0.10 at weeks 4 and 8, respectively, P > 0.05 for both), suggesting that the relative intensity of the exercise may affect PPARγ ligand generation. In untrained individuals, specific transient increases in monocyte expression of PPARγ-regulated genes were observed within 1.5–3 h of exercise (1.7 ± 0.4, 2.6 ± 0.4, and 1.4 ± 0.1 fold for CD36, liver X receptor-α, and ATP-binding cassette subfamily A member 1, respectively, P < 0.05), with expression returning to basal levels within 24 h. In contrast, by the end of the exercise program, expression at the protein level of PPARγ target genes had undergone sustained increases that were not associated with an individual exercise bout (e.g., week 8 Pre/week 0 Pre = 2.79 ± 0.61 for CD36, P < 0.05). Exercise is known to upregulate PPARγ-controlled genes to induce beneficial effects in skeletal muscle (e.g., mitochondrial biogenesis and aerobic respiration). We suggest that parallel exercise-induced effects may occur in monocytes, as monocyte PPARγ activation has been linked to beneficial antiadipetic effects (e.g., exercise-induced upregulation of monocyctic PPARγ-controlled genes is associated with reverse cholesterol transport and anti-inflammatory effects). Thus, exercise-triggered monocyte PPARγ activation may constitute an additional rationale for prescribing exercise to type 2 diabetes patients.

peroxisome proliferator-activated receptor-γ; exercise training; monocytes; reverse cholesterol transport

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With specific regard to monocytes, PPARγ has been reported to regulate inflammatory responses and atherosclerosis via this cell type (20, 39, 46). Importantly, because of differentiation of monocytes into macrophages and their subsequent migration into a wide variety of tissues, activation of PPARγ within monocytes/macrophages has been shown to be crucial to many systemic thiazolidinedione antidiabetic actions throughout the body (18, 20, 40). In particular, upregulation, within monocytes/macrophages, of PPARγ-regulated genes involved in lipid uptake and clearance and reverse cholesterol transport has been shown to promote lipid clearance and transport to the liver within HDL-cholesterol (5, 9, 29).

However, thiazolidinediones such as rosiglitazone have been linked to negative patient outcomes; specifically, the publication of meta- and teleanalyses of clinical rosiglitazone studies (38, 47, 50) has raised safety concerns regarding the increased risk of myocardial infarction and death from cardiovascular causes in rosiglitazone-treated patients. Therefore, because of the immense clinical importance of T2D and its related conditions in Western society, alternative means of inducing beneficial PPARγ-dependent genomic effects without also causing such deleterious effects are urgently required.

Exercise may provide such an alternative: we and others have demonstrated that participation in exercise programs can affect PPARγ-mediated signaling events to bring about antiatherogenic benefits over and above improvements in cardiovascular performance (7, 56, 57). For example, sedentary participants in a low-intensity exercise program (walking 10,000 steps 3 times per week for 8 wk) exhibited increased monocyte PPARγ DNA-binding activity and enhanced expression within monocytes of the PPARγ target genes CD36, PPARγ coactivator-1α (PGC-1α), and liver X receptor-α (LXRα) and, consequently, upregulation of the LXRα target genes ATP-binding cassette (ABC) subfamily A member 1 (ABCA1) and ABC subfamily G member 1 (7). In these subjects, beneficial alterations were observed in serum lipid profiles (increased HDL-cholesterol and decreased total cholesterol, LDL-cholesterol, and triglycerides), with increased HDL-cholesterol correlating with PPARγ target gene expression, indicating that exercise had enhanced reverse cholesterol transport via PPARγ-mediated effects within monocytes (7).

As the activity of PPARγ is primarily governed by the generation and/or availability of its ligands (44, 51), it is possible that the above-mentioned effects were due to generation of PPARγ ligands during exercise. Therefore, we aimed to test the hypotheses that exercise is associated with generation of PPARγ ligands in the plasma and that this may activate PPARγ signaling within circulating monocytes, thus providing a mechanism to underpin the exercise-induced antiatherogenic benefits observed in previous studies (7, 56, 57).

**Materials and Methods**

**Materials**

Reagents were obtained from Sigma-Aldrich (Poole, UK) unless stated otherwise.

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**Participant Recruitment and Exercise Procedures**

The present study comprised two phases: 1) an initial analysis of the response to an acute exercise bout (including a 24-h follow-up) and 2) an 8-wk training program comprising multiple exercise bouts (Table 1).

**Recruitment.** In phase 1, a cohort of healthy active untrained individuals (n = 9, 32 ± 8 yr old, 179 ± 11 cm height, 80 ± 11 kg body mass) was recruited. In phase 2, a cohort of healthy active untrained individuals (n = 8, 27.8 ± 6.4 yr old, 175 ± 7 cm height, 67 ± 6 kg body mass) was recruited. In both cases, institutional ethical approval was granted; all participants signed confidential informed consent, health activity questionnaire, and blood collection forms prior to commencing their involvement.

**Maximal testing.** For phases 1 and 2, all participants completed an incremental cycling test to exhaustion on a Monark 824E cycle ergometer, with 30-W increases in power output every 3 min until volitional exhaustion. Heart rate (Polar s810, Polar Electro) and blood lactate and expired gases (Jaeger Oxycon Delta, Erich Jaeger, Hoechberg, Germany) were analyzed continuously throughout the test. All participants achieved at least two of the criteria for attainment of maximal O2 consumption (V\text{\textsubscript{O}2 max}) (17). Individual plots of power vs. submaximal O2 consumption were used to allow calculation of the power output corresponding to 70% V\text{\textsubscript{O}2 max} for each participant (176 ± 56 and 161 ± 41 W for phases 1 and 2, respectively) and, in the case of phase 2, to establish submaximal training intensities for the subsequent training regimen.

**Training procedures and submaximal testing.** In phase 1, a subsequent single 45-min bout of exercise at 70% V\text{\textsubscript{O}2 max} was performed. Blood and saliva samples were collected before and after exercise; additional samples were collected 1.5, 3, and 24 h after completion of the exercise bout. In phase 2, participants were assigned to an 8-wk training program comprising regular supervised training sessions, also with the same cycle ergometer and heart rate analysis (Table 1). The program was designed to be progressive for training volume (17). At weeks 0, 4, and 8 of the training program, participants performed a constant-load 45-min submaximal test at a constant absolute intensity that was equivalent to 70% V\text{\textsubscript{O}2 max} as calculated from the initial incremental maximal test (161 ± 41 W; sessions 1, 11, and 25 in Table 1). This

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**Table 1. Summary of the 8-wk training program**

<table>
<thead>
<tr>
<th>Week</th>
<th>Session No.</th>
<th>Regimen</th>
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<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1 × (45 min at 70% maximal O\text{\textsubscript{2}} uptake)</td>
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<tr>
<td>1</td>
<td>2</td>
<td>2 × (25 min at 65% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<td></td>
<td>3</td>
<td>3 × (10 min at 80% maximal O\text{\textsubscript{2}} uptake; 4 min recovery)</td>
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<td>2</td>
<td>4</td>
<td>1 × (60 min at 60% maximal O\text{\textsubscript{2}} uptake)</td>
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<td>3</td>
<td>5</td>
<td>4 × (10 min at 80% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<tr>
<td>4</td>
<td>6</td>
<td>1 × (50 min at 65% maximal O\text{\textsubscript{2}} uptake)</td>
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<td>7</td>
<td>5</td>
<td>5 × (8 min at 85% maximal O\text{\textsubscript{2}} uptake; 4 min recovery)</td>
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<td>8</td>
<td>2</td>
<td>2 × (20 min at 70% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<tr>
<td>9</td>
<td>2</td>
<td>2 × (20 min at 75% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<td>10</td>
<td>6</td>
<td>5 × (6 min at 85% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<td>11</td>
<td>1</td>
<td>1 × (45 min at 70% maximal O\text{\textsubscript{2}} uptake)</td>
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<td>12</td>
<td>6</td>
<td>1 × (60 min at 75% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<td>13</td>
<td>4</td>
<td>4 × (10 min at 80% maximal O\text{\textsubscript{2}} uptake; 4 min recovery)</td>
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<td>14</td>
<td>3</td>
<td>3 × (15 min at 80% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<td>15</td>
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<td>1 × (60 min at 65% maximal O\text{\textsubscript{2}} uptake)</td>
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<td>16</td>
<td>2</td>
<td>2 × (15 min at 85% maximal O\text{\textsubscript{2}} uptake; 4 min recovery)</td>
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<td>17</td>
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<td>3 × (15 min at 80% maximal O\text{\textsubscript{2}} uptake; 2 min recovery)</td>
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<td>1 × (45 min at 65% maximal O\text{\textsubscript{2}} uptake)</td>
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<td>3 × (15 min at 80% maximal O\text{\textsubscript{2}} uptake; 3 min recovery)</td>
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<td>2 × (25 min at 80% maximal O\text{\textsubscript{2}} uptake; 2 min recovery)</td>
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<td>21</td>
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<td>1 × (50 min at 70% maximal O\text{\textsubscript{2}} uptake)</td>
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<td>8</td>
<td>22</td>
<td>1 × (60 min at 65% maximal O\text{\textsubscript{2}} uptake)</td>
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<td>4 × (10 min at 85% maximal O\text{\textsubscript{2}} uptake; 4 min recovery)</td>
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<td>1 × (45 min at 70% maximal O\text{\textsubscript{2}} uptake)</td>
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trial was performed under fasting conditions [i.e., tests were performed at 9 AM following an overnight (≥14-h) fast]. Venous blood and saliva samples were taken immediately before (Pre) and after (Post) this 45-min trial, and blood lactate, heart rate, and expired gases were analyzed as previously described. On completion of the training program, using the procedures described above, all participants repeated the maximal test.

Isolation of human peripheral mononuclear cells and plasma samples from whole blood. Blood samples from the antecubital vein were obtained via phlebotomy at the time points described above. Heparinized blood (10 ml) was diluted 1:1 in RPMI medium, layered over 10 ml of Histopaque-1077 Ficoll-Hypaque, and centrifuged at 400 g for 20 min.

Plasma samples were aspirated off as supernatants and stored at −80°C for subsequent analysis; an Instrumentation Laboratories IL-300 analyzer (Diamond Diagnostics, Holliston, MA) was used to determine triglyceride, total cholesterol, and HDL-cholesterol levels in the plasma samples. LDL-cholesterol was calculated using the Friedewald equation. All lipid analyses were carried out at the Diabetes Research Network Cymru laboratories (Swansea, UK).

Measurements of plasma levels of reduced glutathione and salivary levels of IgA. Plasma reduced glutathione ([GSH]plasma) and salivary IgA (sIgA) levels were measured as described previously (32, 33) via commercially available ELISA using anti-GSH capture antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies for [GSH]plasma, or anti-IgA capture antibodies and HRP-conjugated secondary antibodies for sIgA (Calbiochem Biosciences, Merck, Nottingham, UK). Saliva samples were collected from a salivette containing a cotton pad placed under the tongue for 2 min, and the cotton pad was centrifuged (300 g for 2 min) to yield liquid saliva. As reported previously (32, 33), all sIgA data were normalized for hydration status by normalization to salivary protein content [estimated using a Bio-Rad protein assay (Bio-Rad Laboratories, Basingstoke, UK)].

Use of plasma samples in PPRE-luciferase reporter assays. Human embryonic kidney (HEK-293) cells were maintained under standard conditions in DMEM (Invitrogen, Glasgow, UK) supplemented with 4 mM glutamine, 10% (vol/vol) FCS, and 100 IU of penicillin-streptomycin. Cells were transiently transfected in 96-well plates (seeded at a density of 2 × 10^4 cells per well) with 100 ng each of PPRE-luciferase reporter construct, PPARγ expression vector, and Renilla reporter plasmid (pRLTK, Promega, Southampton, UK) using Lipofectamine (Invitrogen) according to the manufacturers’ instructions. The PPARγ expression vector was made using a full-length copy of PPARγ cDNA cloned into pcDNA3 expression vector (Invitrogen). The plasmids-Lipofectamine cocktail in OptiMEM solution (Invitrogen) was incubated at room temperature for 30 min prior to addition to the cells. At 24 h after transfection, the medium was replaced with fresh DMEM, and cells were exposed to rosiglitazone (GlaxoSmithKline, Uxbridge, UK), 15-deoxy-Δ12,14-PGF2α (15PGJ2; Calbiochem, Darmstadt, Germany), and 13-hydroxy-9,11-octadecadienoic acid (13-HODE), which are solubilized in DMSO and used at working concentrations of 1, 3, and 5 μM, respectively, while plasma samples (normalized for protein content) were added at 10% (vol/vol) to DMEM (instead of the addition of 10% FCS). Cells were harvested and lysed 24 h later, and luminescence was determined using a Dual Reporter Assay (Promega) according to the manufacturer’s instructions. In each case, the ratio of luciferase to Renilla luminescence was used to normalize luminescence values to transfection efficiency. For the investigation of the extent to which treatment with plasma samples was associated with phosphorylation of PPARγ, phospho-specific Western blot experiments (see below) were carried out as previously described (8); PPARγ was detected in HEK-293 total-protein extracts as a doublet of ~55-kDa (nonphosphorylated PPARγ) and ~60-kDa (phosphorylated PPARγ) bands.

Isolation of RNA and RT-PCR assays. Total RNA was extracted using a RiboPure-Blood kit (Ambion, Huntingdon, UK) according to the manufacturer’s instructions. Briefly, 5 × 10^6 mononuclear cells or monocytes were washed in ice-cold PBS and lysed, and RNA was extracted using acid phenol-chloroform extraction, ethanol precipitation, and resuspension in RNase-free water. RNA was quantified and checked for purity using the ratio of its absorbance at 260 nm to its absorbance at 280 nm (only samples with a ratio >1.8 were deemed suitable for use). RNA samples were stored at −80°C before conversion to cDNA using the High-Capacity cDNA Archive Kit (Applied Biosystems, Warrington, UK) and then stored at −20°C. PPARγ, CD36, LXRα, ABCA1, PGC-1α, cholesterol ester transferase protein (CETP), lecithin-cholesterol acyltransferase (L-CAT), and ApoA1 mRNA expression were analyzed using Fast SYBR Green Master Mix (Applied Biosystems) and compared with expression of GAPDH; primers with the following sequences were designed using Primer Express software (version 2.0, Applied Biosystems): 5′-GGAAGTGATGATGAACAGCAGC-3′ and 5′-GAGACT- GTTGTTGTCTCAGCG-3′ (CD36), 5′-CCGACTACATCTGCCA-CAGT-3′ and 5′-TGAGGGGGATCTGTTCTTCT-3′ (LXRα), 5′-GCACGTAGAAAGATGCTGAAA-3′ and 5′-AGTTCCGGA-GTTTGTTGTA-3′ (ABCA1), 5′-CGTGGCCGCAGATTTGAA-3′ and 5′-CTTTCAATCCGAGGAGATCAC-3′ (PPARγ), 5′-TACGTG- CTCACGGGACACA-3′ and 5′-TGCTTCGTCGAAAAACAGC-3′ (PGC-1α), 5′-CGGCTCCGGCCACATTACACA-3′ and 5′-GAGCCG -GCATGGGCGATTGCC-3′ (CETP), 5′-TGGCTCATTATTTCTGAT-GCCGCA-3′ and 5′-AGTTGCTGCGGTCTGCGT-3′ (PPARγ). Thermal cycling was carried out as follows: initial denaturation for 10 min at 95°C, followed by 40 cycles of 1 s at 95°C and 60 s at 60°C. Relative quantification of genes of interest was calculated using the 2^−ΔΔCt formula, in which ΔΔCt is the difference between the cycle threshold (Ct) value for the gene of interest and Ct value for the housekeeping gene. Data were included only if plots of log(RNA) vs. ΔCt resulted in slopes between −0.1 and +0.1, meaning that amplification efficiencies were approximately equal, and if plots of log(templat)e vs. Ct resulted in slopes of approximately −3.3 (for the housekeeping gene and the gene of interest; an ideal slope of −3.32 represents 100% PCR efficiency).

Western blot experiments. Western blot experiments were carried out as described elsewhere (32, 33). Briefly, total protein extracts from mononuclear cells were prepared by treatment with 100 μl of protein extraction/lysis buffer containing 1 mM protease inhibitor cocktail and 1 mg/ml phosphatase inhibitor (Active Motif), and protein content was estimated using a protein assay (Bio-Rad Laboratories). Samples were normalized for protein content (50 μg of protein in each case) and subjected to SDS-PAGE, transferred to nitrocellulose, and probed with anti-CD36 or anti-PPARγ primary antibody (16 h, 1:1,000 dilution; Cell Signaling Technology, Danvers, MA) and then with HRP-labeled anti-rabbit IgG antibody (2 h, 1:2,000 dilution; Cell Signaling Technology). Proteins of interest were detected via enhanced chemiluminescence as 88-kDa (CD36) or 55- to 60-kDa (PPARγ) immunogenic bands on Western blots. Band intensities were
determined and quantitated using AC-1 BioImaging and VisionWorks LS Software systems (UltraViolet Products, Cambridge, UK).

Statistical Methods

Values are means ± SE. Multiple comparisons were performed using one-way ANOVA, with post hoc analysis performed using Tukey’s method; for nonmultiple comparisons, paired t-tests were used. Significance levels were set at \( P < 0.05 \).

RESULTS

On completion of the 8-wk training study, the participants showed significantly lower heart rate (175 ± 14 and 156 ± 15 beats/min at weeks 0 and 8, respectively, \( P = 0.002 \); Fig. 1A) and blood lactate (5.9 ± 2.8 and 3.2 ± 1.4 mM at weeks 0 and 8, respectively, \( P = 0.02 \); Fig. 1B) responses at the end of the fixed-intensity 45-min trial, suggesting that the program was effective at promoting a training response in submaximal exercise. Similarly, a borderline-significant increase in \( \text{VO}_{2\text{max}} \) was observed (41.7 ± 8.5 and 43.4 ± 6.8 ml·kg\(^{-1}\)·min\(^{-1}\) at weeks 0 and 8, respectively, \( P = 0.08 \)).

\( \text{sIgA} \) significantly decreased to 53.2 ± 12.7% of basal immediately postexercise at week 0 (\( P < 0.05 \)); however, by week 4, the exercise-associated decrease in \( \text{sIgA} \) was nonsignificant (72.2 ± 17.4% of basal, \( P = 0.232 \)), and by week 8, the \( \text{sIgA} \) postexercise response was further diminished (88.1 ± 18.8% of basal, \( P = 0.550 \); Fig. 1C). Similarly, the same acute bout of exercise was associated with a significant decrease in \([\text{GSH}]_{\text{plasma}}\) in untrained participants (week 0 Post = 68.1 ± 19.1% of week 0 Pre, \( P = 0.004 \); Fig. 1D). However, after 4 wk of training, this exercise-associated decrease in \([\text{GSH}]_{\text{plasma}}\) was blunted (week 4 Post = 76.4 ± 13.9% of week 4 Pre, \( P = 0.016 \)), and after 8 wk of training, there was no significant change in \([\text{GSH}]_{\text{plasma}}\) following an acute bout of exercise (week 8 Post = 101.4 ± 13.7% of week 8 Pre, \( P = 0.846 \)).

Thus, the data shown in Fig. 1 support the hypothesis that several systemic parameters, including physiological (heart rate), metabolic (lactate production), immunological (\( \text{sIgA} \) production), and biochemical (consumption of \([\text{GSH}]_{\text{plasma}}\), which reduces blood-borne reactive oxygen species to harmless forms while being converted to oxidized glutathione), were involved in training adaptations to the 8-wk exercise program.

To determine whether exercise was associated with generation of blood-borne PPAR\( \gamma \) ligands, PPRE-luciferase gene reporter assays were performed. As expected, treatment of HEK-293 cells with the synthetic PPAR\( \gamma \) ligand rosiglitazone (1 \( \mu \)M for 24 h) induced strong PPRE-luciferase activity in cells that had been transfected with a PPAR\( \gamma \) expression vector (3.41 ± 0.30 and 10.52 ± 0.51 relative light units (RLU) for control and rosiglitazone-treated cells, respectively, \( P = 0.05 \); Fig. 2A). In contrast, PPRE-luciferase activity was negligible in the presence or absence of rosiglitazone in cells not transfected with the PPAR\( \gamma \) expression vector (2.04 ± 0.09 and 2.12 ± 0.09 RLU for control non-PPAR\( \gamma \)-transfected cells and...
rosiglitazone-treated non-PPARγ-transfected cells, respectively, \(P < 0.05\) vs. respective PPARγ-transfected samples; Fig. 2A), indicating that this cell type does not express endogenous PPARγ. Treatment with the natural PPARγ ligands 15dPGJ_{2} (14) and 13-HODE (37, 55) also induced increases in PPRE-luciferase activity (9.90 ± 1.06 and 4.80 ± 0.35 RLU for 15d-PGJ_{2} and 13-HODE, respectively, \(P < 0.05\) vs. control in both cases), albeit less strongly than rosiglitazone.

Treatment of PPARγ-expressing HEK-293 cells with week 0 plasma samples [10% (vol/vol) for 24 h] showed increased PPARγ/PPRE-luciferase activity; importantly, this increase was significantly greater when cells were treated with plasma collected following the initial exercise bout (week 0 Post = 123.4 ± 9.7% of week 0 Pre, \(P < 0.05\); Fig. 2B). In contrast, PPRE-luciferase activity was negligible in plasma-treated cells not transfected with the PPARγ expression vector (1.16 ± 0.09 RLU, \(P < 0.05\) vs. respective PPARγ-transfected samples; Fig. 2A). Thus, these data suggest that PPARγ ligands were generated in the plasma as a result of exercise. Interestingly, this effect was blunted in weeks 4 and 8 of the exercise program (week 4 Post = 118.2 ± 13.6% of week 4 Pre and week 8 Post = 109.5 ± 9.6% of week 8 Pre, \(P > 0.05\) in both cases; Fig. 2B).

Western blotting was used to assess PPARγ expression and phosphorylation (8). While PPARγ could not be detected in non-PPARγ-transfected control samples (data not shown), a doublet of ~55-kDa (nonphosphorylated PPARγ) and ~60-kDa [phosphorylated PPARγ (pPPARγ)] bands was detected in all other samples (Fig. 2C). The relative intensities of these two bands indicate that PPARγ was in a predominantly nonphosphorylated state in all samples (pPPARγ-to-total PPARγ ratio = 0.201, 0.102 ± 0.053, and 0.162 ± 0.161 for control, Pre, and Post, respectively; Fig. 2D). Importantly, treatment with Pre or Post exercise plasma samples did not induce significant differences in the pPPARγ-to-total PPARγ ratio.

For participants in phase 1, samples were taken before (Pre) and immediately after (Post) exercise and also at 1.5, 3, and 24 h after exercise. As with week 0 of the 8-wk program (see above) and as shown in our previous studies (32, 33), this bout of exercise was associated with significant decreases in sIgA and GSHplasma (data not shown, \(P < 0.05\) in both cases). The extended 24-h follow-up period in this experiment revealed downstream signaling consequences of participation in exercise. Significant increases (1.5- to 2.5-fold) in expression of the monocyte PPARγ-regulated genes CD36, LXRα, and ABCA1 (\(P < 0.05\) in all cases) were observed in samples taken within 3 h of exercise (Fig. 3), with gene expression returning to basal levels within 24 h of exercise. Gene expression of PPARγ itself, and also of PGC-1α [which has been reported to be encoded by a PPRE-bearing PPARγ target gene (22)], followed a similar trend, with nonsignificant increases at 1.5 and 3 h. In contrast, analysis of expression of reverse cholesterol transport genes, the expression of which is not reported to be increased by PPARγ (11, 15, 35), showed that L-CAT (Fig. 3F) and CETP (data not shown) did not significantly increase, while ApoA1 mRNA could not be detected (suggesting that this gene is not expressed by monocytes or, at least, that its expression levels are below the sensitivity of the assay systems used in the present study). The lack of response of these non-PPARγ target genes indicates that the effects in CD36, LXRα, and ABCA1 (and possibly also PPARγ and PGC-1α) were specifically induced by exercise-triggered PPARγ signaling, rather than as part of a generalized response to exercise.

Although the data shown in Fig. 3A are from mononuclear cells, optimization experiments using CD14+ selection monocyte purification columns (Miltenyi Biotec) showed that...
mRNA expression for the genes of interest in the present study was enriched by two- to sixfold in purified monocytes compared with mononuclear cell samples (5.71 ± 1.09, 2.65 ± 0.16, 2.21 ± 0.58, and 4.62 ± 0.45 for PPARγ, CD36, ABCA1, and PGC-1α, respectively). Importantly, differential expression in monocytes was comparable or greater for each of these genes than the “classical” monocyte marker gene CD14 (2.77 ± 0.47 fold), suggesting that they are predominantly expressed in monocytes, rather than in other types of mononuclear cells.

Western blotting was used to determine expression of PPARγ and its target gene CD36 at the protein level in mononuclear cells (week 0 Pre and week 8 Pre) in participants in phase 2. As shown in Fig. 4, although PPARγ expression underwent only nonsignificant increases (1.50 ± 0.71 at week 8 compared with week 0, *P > 0.05), CD36 expression was
significantly increased at rest (2.79 ± 0.61 at week 8 compared with week 0, P < 0.05; Fig. 4B). No difference in PPARγ phosphorylation was seen between week 0 and week 8 (pPPARγ-to-total PPARγ ratio = 0.231 ± 0.057 and 0.242 ± 0.118, respectively; Fig. 4, C and D). Importantly, the week 8 samples were taken >48 h after the previous bout of exercise, suggesting that any increases in expression were not associated with a single exercise bout but, rather, constituted sustained increases that occurred as a consequence of regular participation in exercise (i.e., 8 wk of adherence to a program comprising regular, repeated exercise; Table 1).

Finally, blood lipids were determined for each participant: at baseline (week 0 Pre), total cholesterol was 4.31 ± 0.20 mM, triglycerides were 1.53 ± 0.19 mM, HDL-cholesterol was 1.33 ± 0.09 mM, and LDL-cholesterol was 2.29 ± 0.17 mM. Thus, all data fell within “healthy/low-risk” categories [American Heart Association reference ranges (3)] for each parameter. A single submaximal standardized exercise bout did not elicit significant changes in blood lipids; also, when comparisons were made between blood lipid levels over the duration of the exercise program, significant decreases in triglycerides (68.0 ± 13.4%, P < 0.05) and borderline-significant decreases in total cholesterol (86.6 ± 17.0%, P < 0.10), but no significant changes in LDL- or HDL-cholesterol, were seen.

**DISCUSSION**

We present data that support the widely held view that exercise is associated with beneficial effects, and we demonstrate several monocyte-associated exercise-induced PPARγ signaling events. The aim of the present study was to elucidate the PPARγ signaling events triggered by exercise and, particularly, by regular exercise training, for example, the downstream effects in terms of gene expression patterns within monocytes and their likely accompanying functional consequences with regard to antiatherogenic and/or anti-inflammatory effects.

It is well established that, in skeletal muscle, exercise up-regulates PPARγ-controlled genes to promote mitochondrial biogenesis, aerobic respiration, and other exercise-triggered benefits. For example, exercise leads to increases in PPARγ activity within skeletal muscle (42), while comparable ~2.5-fold increases in PPARγ expression and, also, expression of the PPARγ coactivator PGC-1α were seen in two separate studies 3 h after a single bout of exercise (16, 30). Moreover, a transcriptional map of the effects of exercise on skeletal muscle has recently been published, and many PGC-1α/PPARγ-regulated genes (e.g., CD36) were among those up-regulated (25). However, if exercise is a systemic phenomenon, its effects will not be limited to a single tissue; therefore, we investigated whether parallel exercise-associated benefits may potentially occur in monocytes via exercise-induced up-regulation of PPARγ-controlled monocytyc genes.

In contrast to skeletal muscle, exercise-related signaling events in monocytes have not been so intensively studied. To our knowledge, this study is novel, in that it demonstrates that exercise is associated with increased levels of PPARγ ligands in the plasma and that PPARγ signaling can be triggered by exercise to exert beneficial effects in circulating monocytes. In light of the data presented in Fig. 2, we propose that generation of PPARγ ligands in the blood during exercise may lead to PPARγ activation in circulating monocytes and, hence, up-regulation of PPARγ target genes (Figs. 3 and 4). As mentioned above, it is possible that the up-regulation of PPARγ-regulated genes in the present study may be due to ligand-independent alteration of PPARγ transcriptional activity. Several studies have demonstrated that receptor-mediated events may lead to changes in PPARγ’s phosphorylation status and, hence, to its activation or inactivation (6, 23); for example, exercise-associated alterations in blood-borne levels of insulin and/or in the ability of insulin to bring about its downstream effects (e.g., the impact of exercise-induced inflammation on insulin receptor substrate-1 tyrosine phosphorylation and, thus, the extent to which insulin receptor occupancy is transduced to downstream kinase activity) have been reported (reviewed in Ref. 26). Also, it is possible that exercise may be associated with a physical change that could impact PPARγ; for example, the rate of blood flow has been reported to influence PPARγ activation via ERK isoforms in endothelial cells (1), an effect that may be mirrored in circulating monocytes during exercise. Importantly, the above-mentioned effects could lead to monocyte PPARγ activation that is not dependent on generation of PPARγ ligands. However, phospho-specific Western blot experiments (8) suggest that treatment of PPARγ-expressing HEK cells with plasma from exercising participants did not increase PPARγ phosphorylation and, also, that there was no difference between “preexercise” and “postexercise” samples with regard to PPARγ phosphorylation (Fig. 2, C and D).

It should be stressed that HEK cells may not express all the signaling agents that might influence PPARγ phosphorylation; therefore, this experimental system may not accurately reflect the in vivo situation in all respects. Nevertheless, we conclude that while exercise-induced PPARγ activation may involve a ligand-independent component in vivo that we could not detect in our experimental system, the specific exercise-associated effects in Fig. 2 are likely to be due to the generation of ligands in the plasma of exercising individuals and subsequent ligand-dependent PPARγ activation within plasma-treated HEK cells. Several lipid-derived agents have been identified as PPARγ ligands (e.g., prostaglandins such as 15dPGJ2 (14) and unsaturated fatty acids such as 13-HODE (37, 55)), and as exercise is associated with changes in lipid metabolism (28), it seems plausible that novel lipid metabolites could be generated within the blood of exercising participants [perhaps due to release of the products of cyclooxygenase and/or lipoxygenase-catalyzed reactions from circulating leukocytes (24)]. In this context, treatment with 15dPGJ2 or 13-HODE induced significant increases in PPRE-luciferase activity in our reporter gene assay system (Fig. 2A). However, precise identification of the putative exercise-generated PPARγ ligands is beyond the scope of the present study.

Previous reports have linked upregulation of PPRE-bearing monocyteic genes with a variety of beneficial functional effects. For example, LXRα, a PPRE-bearing transcription factor analyzed in the present study, controls expression of a number of LXRα target genes that are involved in fatty acid metabolism and cholesterol homeostasis (e.g., ABCA1), which promotes the safe excretion of cholesterol and LDL by formation of Apo-I and HDL particles (9, 29)]. Moreover, upregulation of the scavenger receptor CD36, another PPARγ-regulated gene, leads to increased import of oxidized LDL; processing of
oxidized LDL-derived cholesterol and its LXRα-mediated export within HDL particles may provide an additional link between PPARγ activation and improvements in lipid profiles (7, 9). Finally, the observation that PPARγ activation in monocytes is linked to priming for differentiation within tissues into the M2 macrophage phenotype and also to anti-inflammatory paracrine effects (4, 57) suggests that activation of PPARγ within monocytes/macrophages may be linked to systemic anti-inflammatory actions (18).

However, although parallel exercise-triggered effects may occur in muscle and monocytes, several differences exist between the respective pathways. Specifically, PPARγ exerts different effects through upregulation of a set of target genes in monocytes that is different from that in muscle (possibly due to differences in DNA packaging and/or availabilities of transcriptional coactivators in the respective tissues). However, PPARγ is involved in the systemic regulation and integration of lipid metabolism throughout the body (39). Hence, PPARγ carries out complementary functions in diverse tissues; for example, exercise-triggered PPARγ signaling is involved in stimulation of adipocyte triglyceride hydrolysis, so as to boost release of fatty acids into the bloodstream (52) and also to increase expression of genes linked to oxidative metabolism of fats within skeletal muscle (30). Thus, although different genes are activated in different tissues, a complementary array of effects is induced, and the systemic impact is broadly beneficial. Our study’s identification of monocytes as an exercise-responsive tissue, in which PPARγ can be activated to upregulate genes such as LXRα, CD36, PGC-1α, and ABCA1, is in line with this concept.

In the present study, upregulation of monocyte-expressed PPARγ target genes was seen at the mRNA level <3 h after a single bout of exercise (Fig. 3), but this effect persisted for <24 h. In contrast, after the 8-wk program, increases in PPARγ target gene expression were seen at the protein level in samples taken >48 h after the previous bout of exercise, suggesting that, in this case, the increase is not due to an acute response to an exercise bout. Thus, the following question is raised: can the effects of each individual exercise bout “coalesce,” so that, after an extended program of regular frequent exercise, a more sustained effect is evident? Further experiments are needed to investigate this possibility more fully.

In the present study, these PPARγ signaling effects were associated with decreases in total cholesterol and triglycerides, but not with significant changes in HDL- or LDL-cholesterol. Because of the intensity of the exercise program to be undertaken, the participants recruited into the present study were fit, active individuals. It was not surprising that they exhibited healthy/low-risk values with regard to all four of the lipid parameters studied (3); however, one may speculate that, in cohorts of individuals with suboptimal blood lipid profiles, exercise-induced upregulation of monocyte lipid-handling genes would function to bring lipid levels to within the healthy/low-risk range. In previous studies where participants did not exhibit healthy/low-risk values (3) for these parameters at baseline, larger statistically significant HDL- and LDL-cholesterol effects were seen (7, 56, 58); in our previous study, exercise-induced changes in HDL-cholesterol significantly correlated with Pre vs. Post changes in PPARγ target gene mRNA expression (7), while exercise (sometimes in combination with dietary interventions) has been shown to normalize blood lipid profiles in studies carried out on hypercholesterolemic populations (31). While we acknowledge that this issue and others, such as the nature and small size of the cohort (and the accompanying data variability), are limitations to the present study, ongoing studies within our research group are investigating the effects of exercise on larger cohorts and cohorts of at-risk individuals with suboptimal lipid profiles. In particular, we aim to establish whether the PPRE-luciferase system responds similarly when treated with plasma samples from T2D and non-T2D cohorts, given that similar exercise responses have been shown in T2D and non-T2D cohorts (albeit with regard to skeletal muscle, rather than monocytes) in previous exercise studies (16, 43).

It is well established that regular training influences a range of inflammatory markers (12, 49, 53) in a manner sensitive to exercise intensity and duration (13, 54). The standard response to any effective training program is that the same absolute intensity elicits a reduced physiological perturbation compared with the pretraining response. By presenting diminished postexercise increases in blood-borne PPARγ ligands over the course of the 8-wk program, alongside the expected blunting of postexercise heart rate, lactate, sIgA, and [GSH]plasma responses over the same period, the present work has provided an additional parameter by which a training program may reduce the relative stimulus of a given standardized acute exercise bout. These observations are entirely predictable, given the usual principles of training planning whereby ever-greater absolute intensities are needed to provide sufficient disruption of homeostasis to allow for continued training adaptations (36, 51). However, they are, to our knowledge, novel in their application of this principle to exercise-induced PPARγ signaling. Importantly, given the widely acknowledged benefits of exercise-induced PPARγ signaling, they provide additional support to the concept of a need to tailor exercise programs to participants’ fitness levels to elicit optimally beneficial effects in each case. Thus, while relatively intense exercise was required to activate PPARγ signaling in the cohort of fit, active individuals under investigation in the present study, we and others have reported that low/moderate-intensity exercise (i.e., training programs based on brisk walking) is sufficient to activate PPARγ in cohorts of sedentary individuals (7, 56–58).

It has been well established for several years that exercise is associated with a multitude of benefits, including the prevention and/or treatment of metabolic disorders such as T2D (2). While the present study involved healthy participants and, thus, detected few overt improvements in lipid profiles, individuals with T2D exhibit decreased PPARγ/PGC-1α (34, 41) and dyslipidemia (2). Therefore, if individuals with T2D were to respond in a manner similar to the participants in the present study, and T2D and non-T2D cohorts have been shown to respond to a similar extent to exercise (16, 43), it is possible that the exercise-associated effects seen here could help normalize PPARγ signaling and, thus, improve the blood lipid profiles of the individuals. Thus, the effects described here could potentially contribute to prevention of the development of metabolic disorders such as T2D in healthy or at-risk individuals or to normalization of aberrant metabolic function in individuals with disorders such as T2D.

In conclusion, the present study presents evidence that exercise-associated generation of PPARγ ligands activates PPARγ signaling events that result in upregulation of mono-
cytic PPARγ target genes. This may constitute a novel beneficial effect of exercise in the context of prevention and/or treatment of metabolic disorders such as T2D and its complications.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


