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

A. W. Thomas,1,* N. A. Davies,1* H. Moir,1 L. Watkeys,1 J. S. Ruffino,1 S. A. Isa,1 L. R. Butcher,1 M. G. Hughes,2 K. Morris,1 and R. Webb1

1Cardiff School of Health Sciences and 2Cardiff School of Sport, University of Wales Institute Cardiff, Cardiff, United Kingdom

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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 antiadipogenic effects (e.g., exercise-induced upregulation of monocytic 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.

* A. W. Thomas and N. A. Davies contributed equally to this work.

Address for reprint requests and other correspondence: R. Webb, Cardiff School of Health Sciences, UWIC, Cardiff CF5 2YB, UK (e-mail: rwebb@uwic.ac.uk).

The recent joint position statement released by the American College of Sports Medicine and the American Diabetes Association stated that “it is now well established that participation in regular physical activity can... prevent or delay Type 2 Diabetes (T2D), and positively affect lipids, blood pressure, cardiovascular events, mortality and quality of life” (2). During exercise, energy demand increases dramatically in skeletal muscle; consequently, skeletal muscle was among the first tissues to be investigated with regard to responses to exercise (21). However, the diversity of the effects listed above indicates that exercise is a systemic phenomenon with impact that is not limited to one tissue. Indeed, more recent research has shown that signaling events within a wide range of cell types are affected by exercise (52).

The present study focuses on the effects of exercise on monocytes, which are important in several aspects of the pathophysiology of T2D, including cardiovascular complications such as atherosclerosis [with a link to T2D that is largely due to the proatherogenic nature of glycated proteins and lipids (48)]. Monocytes have roles in control of blood-borne lipid homeostasis via their contribution to reverse cholesterol transport (9, 29) and in paracrine control of inflammatory signaling (4); also, adherence of monocytes to the endothelial wall and their migration into the subendothelial space and differentiation into macrophage foam cells are crucial to the development of atherosclerosis (48). The contribution of monocyte-mediated reverse cholesterol transport to healthy lipid homeostasis is a major focus of the present study: after uptake by monocytes, lipoprotein components are processed and presented to apolipoprotein A-1 (ApoA1), thus forming HDL-cholesterol, which acts to reduce cardiovascular risk, as cholesterol bound to this lipoprotein is transported to the liver for safe disposal (5, 9, 29). This may be of considerable clinical importance: selective activation of this pathway in peripheral cells such as monocytes has been recommended as a potential therapeutic target for atherosclerosis (5).

The ligand-activated nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) is a regulator of the expression of numerous PPARγ target genes [i.e., genes bearing PPAR response elements (PPREs), to which activated PPARγ can bind] involved in metabolism, cell differentiation, and inflammation (44, 51). Thus, the actions of synthetic agonists for PPARγ [e.g., thiazolidinediones such as rosiglitazone (27)] in regulating PPARγ target gene expression are responsible for beneficial effects in the context of T2D (10). However, the transcriptional activity of PPARγ may also be affected by its phosphorylation status, and kinases such as MAPK/ERK, AMP-activated protein kinase, and others have been associated with ligand-independent changes in PPARγ activity (6).
With specific regard to monocytes, PPARγ has been reported to regulate inflammatory responses and atherogenesis 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-meditated signaling events to bring about anti-inflammatory effects due to upregulation of PGC-1α and priming of monocytes for differentiation into the alternative M2 macrophage phenotype (57).

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.

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 Oxycron 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 (VO2max) (17). Individual plots of power vs. submaximal O2 consumption were used to allow calculation of the power output corresponding to 70% VO2max 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% VO2max 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% VO2max, as calculated from the initial maximal test (161 ± 41 W; sessions 1, 11, and 25 in Table 1). This

Table 1. Summary of the 8-wk training program

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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).

Mononuclear cell fractions were carefully removed from the Ficoll-Hypaque interface and washed four times (500 g for 10 min) in 0.4 ml of phosphatase inhibitor solution (Active Motif, Rixensart, Belgium) and 7.6 ml of PBS. For validation purposes, direct-labeling monocyte purification experiments were carried out in some cases. Mononuclear cells were incubated with CD14-conjugated magnetic microbeads [Miltenyi Biotec, Surrey, UK; 1:5 (vol/vol) at 4°C for 15 min]. Excess microbeads were removed by centrifugation (300 g for 10 min) and removal of the resulting supernatant. Cells were passed through a 30-μm-pore filter and added to a separator column (MACS LS, Miltenyi Biotec) attached to a magnetic separator unit (QUADROMACS). The column was washed to remove unbound cells and removed from the magnetic separator unit, and cells were forced through the column with a syringe plunger to elute labeled cells (i.e., CD14+ monocytes).

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 × 104 cells per well) with 100 ng each of PPRE-luciferase reporter construct, PPARγ expression vector, and Renilla reporter plasmid (pR6TK, Promega, Southhampton, 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 resiglitazone (GlaxoSmithKline, Uxbridge, UK), 15-deoxy-Δ12,14-PGJ2 (15PGJ2; Calbiochem, Darmstadt, Germany), and 13-hydroxy-9,11-octadecadienoic acid (13-HODE), which were 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 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, Huntington, 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, LRxRα, ABCA1, PGC-1α, cholesteryl ester transferase protein (CETP), lecithin-cholesterly 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- GTTGTTGTCCTCACGG-3′ (CD36), 5′-GGCACTACATCTGCACT CAGT-3′ and 5′-TGAGGGGATCTGGTTCTTCT-3′ (LRxRα), 5′- GACATGGAAGATGCTGAAA-3′ and 5′-AGTTCCTGGAG GTTTGTTGCA-3′ (PPARγ), 5′-CTTCATCCTGAGGAGAAGTTCAACA-3′ and 5′-5′-GACAGC-GCATGGGCGATTGC-3′ (CETP), 5′-CCCTGTGCCTGCAACTACAGG-3′ (PGC-1α), 5′-CGGCTCCTGGGACCATTCACAACA-3′ and 5′-GACAGC-GCATGGGCGATTGC-3′ (CETP), 5′-TGTCATATTTCGCTT GGCCCA-3′ and 5′-ATGGGGCATCTGCTGGTTC-3′ (L-CAT), 5′-ECCCTACAGGGCACGGACTG-3′ and 5′-GTTGCGCTT- GGCCCTGTTACT-3′ (ApoA1), and 5′-CTCTCTGGGACTCA CGAA-3′ and 5′-GAGACGATTTCGGCGTGGAC-3′ (GAPDH). Thermocycling was carried out as follows: initial denaturation for 10 min at 95°C, followed by 40 cycles of 15 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 amplicon efficiencies were approximately equal, and if plots of log(template) vs. Ct resulted in slopes of approximately −3.3 (for the housekeeping gene and the gene of interest; an ideal slope of −3.3 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 [GSH]plasma 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).

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 sIgA was nonsignificant (77.2 ± 17.4% of basal, *P* = 0.232), and by week 8, the 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 [GSH]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 [GSH]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 [GSH]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 (sIgA production), and biochemical (consumption of [GSH]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 PPARγ ligands, PPRE-luciferase gene reporter assays were performed. As expected, treatment of HEK-293 cells with the synthetic PPARγ ligand rosiglitazone (1 μM for 24 h) induced strong PPRE-luciferase activity in cells that had been transfected with a PPARγ 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 PPARγ expression vector (2.04 ± 0.09 and 2.12 ± 0.09 RLU for control non-PPARγ-transfected cells and

![Fig. 1. Training adaptations: responses to a 45-min bout of cycling at 70% of maximal O2 uptake (V̇O2 max), as observed during an 8-wk exercise program.](http://japp.physiology.org/)
Peroxisome proliferator-activated receptor (PPAR) response element (PPRE)-luciferase activity and PPARγ phosphorylation, as determined in human embryonic kidney (HEK-293T) cells treated with plasma samples from exercising participants. PPRE-luciferase activity was determined in HEK-293T cells [with and without (+ and −) PPARγ transfection] treated for 24 h with 1 μM rosiglitazone, 3 μM 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2), 5 μM 13-hydroxy-9,11-octadecadienoic acid (13-HODE), or plasma samples [10% (vol/vol)] obtained before (Pre) and after (Post) exercise (A) or Pre (open bars) and Post (filled bars) plasma samples obtained at weeks 0, 4, and 8 of an exercise program (B). RLU, relative light units. C and D: representative images and densitometric data from Western blot experiments in which total protein extracts from plasma-treated HEK-293T cells were probed for phosphorylated PPARγ (pPPARγ) and nonphosphorylated PPARγ (~60 and ~55 kDa, respectively). Values are means ± SE (n = 8). *P < 0.05.

Fig. 2. Peroxisome proliferator-activated receptor (PPAR) response element (PPRE)-luciferase activity and PPARγ phosphorylation, as determined in human embryonic kidney (HEK-293T) cells treated with plasma samples from exercising participants. PPRE-luciferase activity was determined in HEK-293T cells [with and without (+ and −) PPARγ transfection] treated for 24 h with 1 μM rosiglitazone, 3 μM 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2), 5 μM 13-hydroxy-9,11-octadecadienoic acid (13-HODE), or plasma samples [10% (vol/vol)] obtained before (Pre) and after (Post) exercise (A) or Pre (open bars) and Post (filled bars) plasma samples obtained at weeks 0, 4, and 8 of an exercise program (B). RLU, relative light units. C and D: representative images and densitometric data from Western blot experiments in which total protein extracts from plasma-treated HEK-293T cells were probed for phosphorylated PPARγ (pPPARγ) and nonphosphorylated PPARγ (~60 and ~55 kDa, respectively). Values are means ± SE (n = 8). *P < 0.05.

The 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). Hence, 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 [GSH]plasma (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

![Fig. 3. Effect of exercise on gene expression in mononuclear cells.](image)

![Fig. 4. Effect of exercise on CD36 and PPARγ expression in mononuclear cells.](image)
significantly increased at rest (2.79 ± 0.61 at week 8 compared with week 0, \( P < 0.05 \); Fig. 4B). No difference in PPAR\(\gamma\) phosphorylation was seen between week 0 and week 8 (pPPAR\(\gamma\)-to-total PPAR\(\gamma\) 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 PPAR\(\gamma\) signaling events. The aim of the present study was to elucidate the PPAR\(\gamma\) 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 PPAR\(\gamma\)-controlled genes to promote mitochondrial biogenesis, aerobic respiration, and other exercise-triggered benefits. For example, exercise leads to increases in PPAR\(\gamma\) activity within skeletal muscle (42), while comparable ~2.5-fold increases in PPAR\(\gamma\) expression and, also, expression of the PPAR\(\gamma\) coactivator PGC-1\(\alpha\) 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-1\(\alpha\)/PPAR\(\gamma\)-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 PPAR\(\gamma\)-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 PPAR\(\gamma\) ligands in the plasma and that PPAR\(\gamma\) 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 PPAR\(\gamma\) ligands in the blood during exercise may lead to PPAR\(\gamma\) activation in circulating monocytes and, hence, up-regulation of PPAR\(\gamma\) target genes (Figs. 3 and 4). As mentioned above, it is possible that the up-regulation of PPAR\(\gamma\)-regulated genes in the present study may be due to ligand-independent alteration of PPAR\(\gamma\) transcriptional activity. Several studies have demonstrated that receptor-mediated events may lead to changes in PPAR\(\gamma\)'s phosphorylation status and, thence, 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 PPAR\(\gamma\); for example, the rate of blood flow has been reported to influence PPAR\(\gamma\) 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 PPAR\(\gamma\) activation that is not dependent on generation of PPAR\(\gamma\) ligands. However, phospho-specific Western blot experiments (8) suggest that treatment of PPAR\(\gamma\)-expressing HEK cells with plasma from exercising participants did not increase PPAR\(\gamma\) phosphorylation and, also, that there was no difference between “preexercise” and “postexercise” samples with regard to PPAR\(\gamma\) phosphorylation (Fig. 2, C and D).

It should be stressed that HEK cells may not express all the signaling agents that might influence PPAR\(\gamma\) phosphorylation; therefore, this experimental system may not accurately reflect the in vivo situation in all respects. Nevertheless, we conclude that while exercise-induced PPAR\(\gamma\) 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 PPAR\(\gamma\) activation within plasma-treated HEK cells. Several lipid-derived agents have been identified as PPAR\(\gamma\) ligands [e.g., prostaglandins such as 15dPGJ\(_2\) (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 15dPGJ\(_2\) 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 PPAR\(\gamma\) ligands is beyond the scope of the present study.

Previous reports have linked upregulation of PPRE-bearing monocytyc genes with a variety of beneficial functional effects. For example, LXR\(\alpha\), a PPRE-bearing transcription factor analyzed in the present study, controls expression of a number of LXR\(\alpha\) 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 PPAR\(\gamma\)-regulated gene, leads to increased import of oxidized LDL; processing of...
oxidized LDL-derived cholesterol and its LXRx-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 (50). 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 LXRx, 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 monocytic lipid-handling genes would function to bring lipid levels to within the healthy/low-risk range. In previous studies where participants did not 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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