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Adding exercise to rosuvastatin treatment: influence on C-reactive protein, monocyte toll-like receptor 4 expression, and inflammatory monocyte (CD14+CD16+) population

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Abstract

Statin treatment and exercise training can reduce markers of inflammation when administered separately. The purpose of this study was to determine the effect of rosuvastatin treatment and the addition of exercise training on circulating markers of inflammation including C-reactive protein (CRP), monocyte toll-like receptor 4 (TLR4) expression, and CD14+CD16+ monocyte population size. Thirty-three hypercholesterolemic and physically inactive subjects were randomly assigned to rosuvastatin (R) or rosuvastatin/exercise (RE) groups. A third group of physically active hypercholesterolemic subjects served as a control (AC). The R and RE groups received rosuvastatin treatment (10 mg/d) for 20 weeks. From week 10 to week 20, the RE group also participated in an exercise training program (3d/wk). Measurements were made at baseline (Pre), week 10 (Mid), and week 20 (Post), and included TLR4 expression on CD14+ monocytes and CD14+CD16+ monocyte population size as determined by 3-color flow cytometry. Serum CRP was quantified by enzyme-linked immunosorbent assay. TLR4 expression on CD14+ monocytes was higher in the R group at week 20. When treatment groups (R and RE) were combined, serum CRP was lower across time. Furthermore, serum CRP and inflammatory monocyte population size were lower in the RE group compared with the R group at the Post time point. When all groups (R, RE, and AC) were combined, TLR4 expression was greater on inflammatory monocytes (CD14+CD16+) compared with classic monocytes (CD14+CD16−) at all time points. In conclusion, rosuvastatin may influence monocyte inflammatory response by increasing TLR4 expression on circulating monocytes. The addition of exercise training to rosuvastatin treatment further lowered CRP and reduced the size of the inflammatory monocyte population, suggesting an additive anti-inflammatory effect of exercise.

1. Introduction

Statins (hydroxymethylglutaryl–coenzyme A reductase inhibitors) are the most effective pharmacologic intervention for hypercholesterolemia [1]. Statins have also been reported to attenuate chronic low-grade inflammation [2]. This was initially evidenced in large clinical trials by lower serum C-reactive protein(CRP) after treatment [3,4]. Although the specific mechanisms by which statin treatment elicits this effect have yet to be elucidated, a number of reports suggest that statin treatment alters the production of inflammatory cytokines from monocyte/macrophage. For example, pretreatment of cultured macrophage with statins augment lipopolysaccharide (LPS)-stimulated production of interleukin (IL)-1α, IL-1β, and IL-6 [5-8]. However, some reports have reached the opposite conclusion and suggest that statins suppress LPS activation of cultured monocytes [9] and reduce LPS activation of nuclear factor–κB [10,11]. Furthermore, in patients with hypercholesterolemia, simvastatin inhibited monocyte expression of proinflammatory cytokines tumor necrosis factor (TNF)–α, IL-1β, and IL-8 [12,13].

Statins may elicit their effects on the monocyte/macrophage by altering the expression of the receptor for LPS, toll-

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like receptor 4 (TLR4). Niessner et al [14] found that simvastatin pretreatment (80 mg/d) blunted the increase in monocyte TLR4 and TLR2 expression in response to LPS infusion [14]. This effect occurred concomitantly with lower circulating levels of TNF-α. Methe et al [15] found that atorvastatin lowered TLR4 expression on cultured human monocytes and lowered LPS-stimulated IL-6 and TNF-α production [15]. In the same study, 4 weeks of atorvastatin treatment in normocholesterolemic subjects reduced TLR4 expression on CD14+ monocytes. Taken together, these studies suggest that statins alter monocyte inflammatory response to LPS by lowering TLR4 expression and may also explain the reported anti-inflammatory effect of statin treatment. Understanding how statin treatment influence inflammation is important because chronic low-grade inflammation underlies many disease states. Specifically, an activated monocyte/macrophage inflammatory response and TLR4 activation contribute to the pathophysiology of obesity, diabetes, and atherosclerosis [16,17].

Exercise training is also known to exert anti-inflammatory effects including reduced serum CRP [18], circulating inflammatory monocyte (CD14+CD16+) percentage [19], and LPS-stimulated whole blood cytokine production [20,21]. A reduction in TLR4 expression is a potential mechanism that mediates the anti-inflammatory effect of exercise training. We previously reported that CD14+ monocyte expression of TLR4 was lower in well-trained compared with untrained elderly women [22]. Furthermore, exercise training reduced TLR4 expression on CD14+ monocytes in previously sedentary young and old subjects and concomitantly reduced LPS-stimulated whole blood production of IL-6 [23].

The influence of statin treatment and exercise training combined on mediators of inflammation has not been studied extensively. Troseid et al [24] found that pravastatin and exercise treatment administered together elicited a more substantial decline in monocyte chemotactic protein−1 compared with pravastatin alone. The purpose of the present study was to examine the effect of rosuvastatin treatment and the addition of an exercise training program on markers/mediators of inflammation. We hypothesized that rosuvastatin treatment would lower TLR4 expression on CD14+ monocytes, serum CRP, and the size of the inflammatory monocyte (CD14+CD16+) population and that the addition of exercise training would elicit a further anti-inflammatory effect.

2. Materials and methods

2.1. Study design

A longitudinal 3 × 3 design was used (3 groups × 3 time points). Thirty-four hypercholesterolemic and physically inactive male (40-65 years old) and female (45-65 years old and postmenopausal) subjects were randomly divided into 2 groups: a rosuvastatin/exercise (RE) and rosuvastatin (R)-only group. Subjects in the R and RE groups received rosuvastatin calcium (10 mg/d) treatment for 20 weeks. From week 10 to week 20, subjects in the RE group completed a 10-week exercise program in addition to their rosuvastatin treatment, whereas subjects in the R group continued rosuvastatin treatment and remained physically inactive. Subjects participating in the RE and R groups required a signed consent letter from their physician and a prescription for rosuvastatin calcium treatment before enrollment. Prescriptions for rosuvastatin calcium were filled at Purdue University pharmacy. A third group of 18 hypercholesterolemic and physically active subjects was recruited for the control group (AC). All subjects were evaluated at baseline (Pre), week 10 (Mid), and week 20 (Post). This study was approved by the Biomedical Institutional Review Board at Purdue University (protocol no. 0505002668).

2.2. Screening

Subjects for this study were recruited through advertisements in the West Lafayette, IN, area. A brief phone screening initially determined eligibility. One week before baseline testing, potential subjects reported to the laboratory in a fasted state for screening and preliminary tests. Potential subjects read and signed an informed consent document, and completed a medical history questionnaire, an inclusion-exclusion criteria questionnaire, and a medications and supplements disclosure form. Potential subjects were excluded if they reported musculoskeletal or orthopedic limitations, previous myocardial infarction or stroke, liver or kidney disease, diabetes mellitus, hypothyroidism, or renal insufficiency. Potential subjects were also excluded if they were taking medications known to directly or indirectly influence leukocyte function or inflammation, other lipid-lowering agents, or drugs known to interfere with statin treatment. Hormone replacement therapies were not exclusionary criteria for postmenopausal women.

After 20 minutes of seated rest, blood was drawn into a serum separator tube; and aliquots of serum were stored at −80°C. Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were quantified for each subject using a standard spectrophotometric method (Infinity Reagents; Thermo Scientific, Waltham, MA). Low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald equation [25]. The upper limit for body mass index (BMI) was 35 kg/m^2. Body fat was estimated by 3-site skinfold measurement [26].

A physical activity questionnaire and Balke submaximal treadmill test to predict maximum oxygen consumption (Vo_{2max}) were used to determine physical activity levels [26,27]. To be included in the AC group, subjects reported exercising at least 3 days per week for the previous 6 months and had a “good” to “superior” estimated Vo_{2max} (women, >28 mL/[kg min]; men, >35 mL/[kg min]). The RE and R subjects reported little or no regular exercise over the previous 6 months and had a “fair” to “very poor” Vo_{2max} (women, <25 mL/[kg min]; men, <32 mL/[kg min]).

Physically inactive subjects with total cholesterol greater than 200 mg/dL and LDL greater than 130 mg/dL were randomly assigned to the R and RE groups. Physically active subjects with total cholesterol greater than 200 mg/dL and LDL less than 160 mg/dL were enrolled in the AC group. The AC subjects were asked to maintain and record their habitual activity level throughout the 20-week study. Subjects in the R and RE groups had a cardiovascular disease risk level that warranted drug treatment, whereas subjects in the AC group did not, as determined by the Adult Treatment Program III guidelines [28].

A total of 102 subjects were screened, and 52 hypercholesterolemic male and female subjects were recruited (Fig. 1). Forty-nine subjects completed the study, and 3 subjects dropped out for personal reasons (2 AC males; 1 RE male).

2.3. Acclimation and exercise training

After 10 weeks of rosuvastatin treatment, subjects in the RE group completed 3 acclimation sessions. Subjects were taught the correct lifting technique for the following exercises: leg press, leg extension, leg curl, chest press, lat pull-down, seated row, leg adduction, and leg abduction (Keiser, Fresno, CA) in the first session. For the second session, each subject's 8 repetition maximum (RM) was determined for each of the resistance exercises. Finally, for the third session, 1 RM was determined for the chest press, leg press, and leg curl.

After acclimation, RE subjects completed 10 weeks (3 d/wk, 30 sessions) of combined endurance and resistive exercise training. The endurance training portion consisted of 20 minutes of treadmill walking at 60% to 70% of heart rate reserve. Heart rate was checked periodically to ensure subjects were exercising at the correct intensity. The subjects then completed a set of stretches and performed 2 sets of 8 resistance exercises. For the first week, subjects completed the resistance exercises at 70% of estimated 1 RM. For subsequent weeks, exercises were performed at 80% of estimated 1 RM. If subjects were able to complete more than 12 repetitions in the second set for 2 consecutive training sessions, the resistance for that exercise was increased for the following session (~5%-10%). Eight RM, 1 RM, and estimated $V_{O_{2max}}$ were reassessed at the end of the training program.

2.4. Trial day procedure

Blood samples were collected at baseline and after 10 and 20 weeks. On the day before each blood draw, subjects

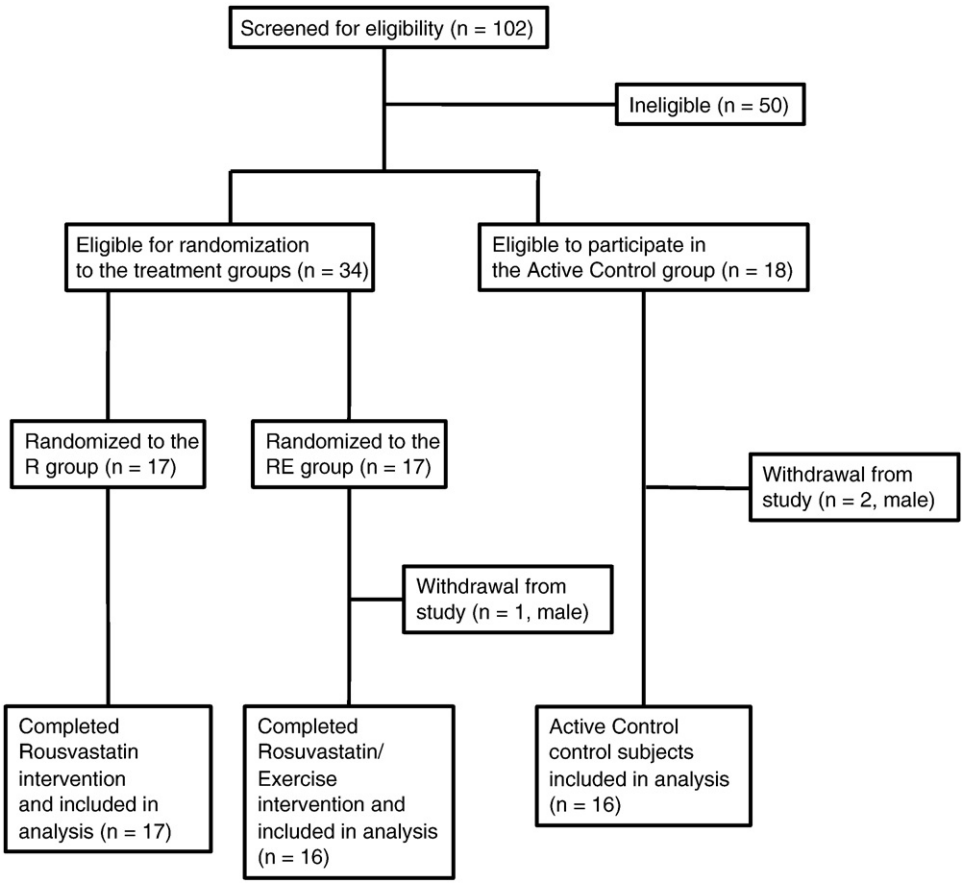

Fig. 1. CONSORT flow diagram depicting progress of all participants through the study.

followed a 1-day diet based on the American Diabetes Association dietary exchange program, which contained 50% carbohydrate, 35% fat, and 15% protein. Subjects reported to the laboratory on testing days between 6:00 and 8:00 AM after an overnight fast and 72 hours removed from their last exercise bout. Blood was drawn as previously described into a serum separator tube and sodium heparin tube (Becton-Dickinson, Franklin Lakes, NJ).

2.5. Monocyte expression of CD14/CD16/TLR4

Monocyte surface expression of TLR4/CD14/CD16 was determined by 3-color flow cytometry (Cytomics FC500; Beckman-Coulter, Fullerton, CA). Briefly, 100 μL of heparinized blood was stained with antibodies specific for human CD14 (CD14-FITC; Biolegend, San Diego, CA), CD16 (CD16-Pe-Cy5, Biolegend), and TLR4 (TLR4-PE, Beckman-Coulter). A separate sample was also stained with appropriate isotype control antibodies and used to establish negative gates. A primary gate was established for monocyte subpopulation based on light scatter (Fig. 2A), with a secondary gate set for CD14+ monocytes. The TLR4 expression relative to isotype control (Fig. 2B) was determined for total CD14+ monocytes and both CD14+ CD16+ and CD14+CD16− monocyte populations. Inflammatory (CD14+CD16+) and classic monocyte (CD14+ CD16−) populations were determined based on CD16 expression on CD14+ monocytes in the secondary gate (Fig. 2C). A total of 100 000 events were acquired for each sample, with approximately 8000 events acquired within the monocyte primary gate. Fluorospheres were used daily to verify the performance of instrument optical alignment and fluidics system (Flow-Check, Beckman-Coulter).

2.6. Serum CRP

Serum CRP concentration was measured by enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH). The inter- and intraassay coefficients of variation for the CRP enzyme-linked immunosorbent assays were less than 10%.

2.7. Power calculation

A power calculation was carried out to determine a sample size that was adequate to detect significant changes in the primary response variables, which were TLR4 expression and inflammatory monocyte percentage. To obtain an estimate of the variability in TLR4 expression, we used data

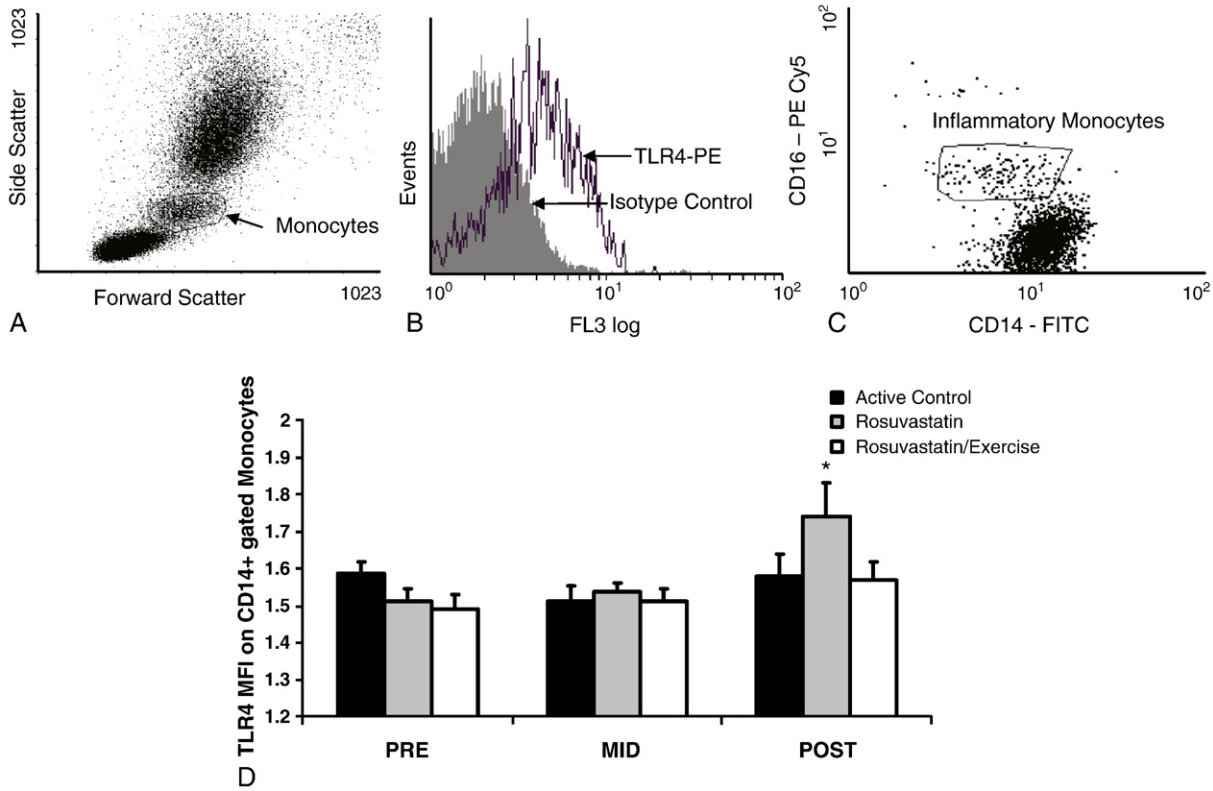

Fig. 2. A, Representative dot plot depicting placement of the primary gate around the monocyte subpopulation based on forward and side light scatter. B, Representative overlay histogram showing the TLR4 positive and isotype control peaks for gated CD14+ monocytes. C, Representative dot plot depicting gate placement around inflammatory monocyte (CD14+CD16+) population. D, TLR4 expression (MFI) on CD14+ monocytes. *The R group was higher at Post compared with Pre ($P = .011$).

Table 1
Descriptive data for study groups

Group	n	Age (y)	Height (cm)	Body mass (kg)		BMI (kg/m^2)		% Body fat	
				Pre	Post	Pre	Post	Pre	Post
AC	9 F, 7 M	51.62 ± 1.09	170.98 ± 2.65	74.14 ± 3.74*	75.07 ± 3.69*	24.66 ± 0.81*	24.82 ± 0.80*	24.81 ± 2.24*	26.05 ± 2.18*
R	8 F, 9 M	52.11 ± 1.45	170.88 ± 3.40	84.95 ± 5.22†	84.41 ± 6.17†	28.51 ± 1.01	28.17 ± 0.95	30.99 ± 1.84	29.32 ± 2.04
RE	9 F, 7 M	52.18 ± 1.29	170.34 ± 2.11	80.37 ± 4.86	80.88 ± 4.76	27.30 ± 1.09	28.07 ± 1.17	30.33 ± 1.95	30.40 ± 1.99

Values are mean ± SE.
* AC (Pre and Post) body mass ($P < .05$), BMI ($P < .0001$), and percentage body fat ($P < .0001$) were lower than R and RE.
† R (Pre and Post) was higher than RE ($P = .0045$).

from a previous study [29]. It was determined that a sample size of 16 would give 80% power to detect a mean difference in TLR4 expression on CD14+ monocytes.

2.8. Statistical analysis

All data are reported as mean ± standard error. Statistical analyses were performed using SAS (Cary, NC) Version 9.0. Before statistical analysis, all data were tested for the assumptions of normality, equality of variance, and independence. A group × time analysis of variance was used to test study hypotheses. A Tukey post hoc test was used to determine if comparisons were significant. Statistical significance was set at $P < .05$.

3. Results

3.1. Subject descriptive data

There were no significant differences in BMI or estimated percentage body fat between the R and RE groups. However, the R group had a higher body mass than the RE group ($P = .0045$) (Table 1). The AC group had lower body mass ($P < .05$), BMI ($P < .001$), and estimated percentage body fat ($P < .0001$) compared with the R and RE groups (Table 1). Within each group, male subjects had a higher body weight ($P < .02$) and lower estimated percentage body fat ($P < .04$) compared with female subjects. There were no significant sex or group changes in weight, BMI, or estimated percentage body fat over time.

3.2. Aerobic fitness and strength

Estimated $V_{O_{2max}}$ was significantly higher in the AC group (Pre, 40.78 ± 1.7 mL/[kg min]; Post, 38.18 ± 1.8 mL/[kg min]) compared with R (Pre, 26.97 ± 1.2; Post, 28.08 ± 1.2 mL/[kg min]) and RE groups (Pre, 26.47 ± 0.9; Post, 34.98 ± 2.3 mL/[kg min]) ($P < .0001$). Within each group, male subjects had a higher estimated $V_{O_{2max}}$ ($P < .04$) compared with female subjects. Estimated $V_{O_{2max}}$ increased significantly in the RE group after exercise training (+29% ± 6%, $P < .0001$). The increase in estimated $V_{O_{2max}}$ was similar for both male and female subjects in the RE group. Muscular strength increased significantly for all exercises in the RE group ($P < .0001$) (8 RM range, 30% ± 4%-57% ± 10%; 1 RM range, 16% ± 3%-20% ± 17%). Within the RE group, male subjects had greater muscular strength for all exercises ($P < .04$) compared with female subjects. Male and female subjects in the RE group increased strength to the same degree after exercise training. The average number of training sessions completed by the RE group was 29 (range, 26-30 sessions).

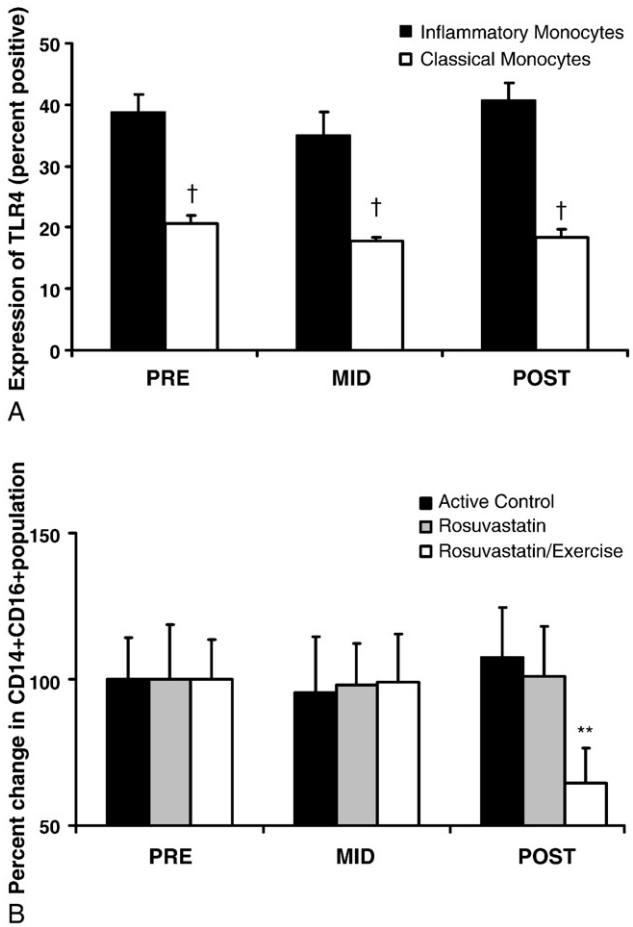

Fig. 3. A, TLR4 expression on inflammatory and classic monocytes populations at each time point (R, RE, and AC groups combined). †The percentage of cells positive for TLR4 expression was higher for inflammatory monocytes compared with classic monocytes ($P < .001$). B, The percentage change in CD16+CD14+ population (Mid and Post values normalized to Pre, which was set to 100%). **The RE group was lower at Post compared with the Mid time point ($P = .013$).

3.3. Expression of TLR4 and inflammatory monocyte population

The level of TLR4 expression (mean fluorescence intensity [MFI]) on CD14+ monocytes was significantly higher in the R group at the Post time point when compared with the Pre time point ($P = .011$, Fig. 2D). The percentage of CD14+ monocytes that expressed TLR4 was approximately 16.6% and was not significantly different between or within groups over the course of the intervention. When study groups were combined (RE, R, and AC), the inflammatory monocytes expressed more TLR4 (percentage positive) compared with classic monocytes at all time points ($P < .001$. Fig. 3A). Inflammatory monocyte percentage was higher for AC (group effect) (Pre, 4.65% ± 0.66%; Mid, 4.45% ± 0.87%; Post, 4.95% ± 0.80%) than the R (Pre, 3.47% ± 0.64%; Mid, 3.40% ± 0.49%; Post, 3.50% ± 0.59%) and RE (Pre, 3.69% ± 0.5%; Mid, 3.65% ± 0.61%; Post, 2.37% ± 0.45%) groups ($P < .05$). The size of the inflammatory monocyte population was lower in the RE group at Post when compared with the Mid time point ($P = .013$, Fig. 3B). There were no sex effects observed for TLR4 expression or inflammatory monocyte population size. There was no significant difference in CD14 expression (percentage positive and MFI) between groups or with the interventions (data not shown).

3.4. Serum levels of CRP

When the RE and R groups were combined, CRP was lower at the Mid ($P = .009$) and Post ($P = .001$) time point when compared with Pre, indicating a primary effect of rosuvastatin treatment (Fig. 4). There was also an interaction effect with the addition of exercise training, such that CRP was lower in the RE group compared with the R group, at the Post time point ($P = .049$, Fig. 4). There were no sex effects observed for serum levels of CRP.

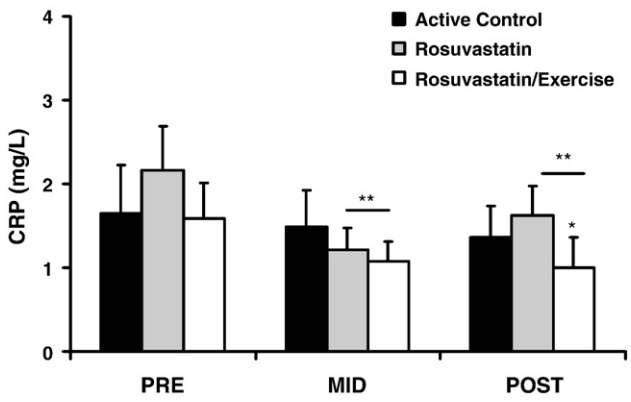

Fig. 4. Serum CRP. **C-reactive protein in the RE and R groups combined was lower at the Mid ($P < .009$) and Post ($P < .001$) time point compared with Pre. *C-reactive protein was lower in the RE group compared with the R group at the Post time point ($P = .049$).

4. Discussion

Statin treatment and exercise training have both been reported to elicit anti-inflammatory effects. Although the mechanisms by which rosuvastatin and exercise elicit their anti-inflammatory effect have not been fully elucidated, an influence on TLR4 expression/activation has been reported for both. The purpose of the present study was to examine the influence of rosuvastatin treatment with and without the addition of an exercise training program on CD14+ monocyte TLR4 expression, the size of the inflammatory monocyte (CD14+CD16+) population, and serum CRP. The key findings from the present study were that TLR4 expression on CD14+ monocytes was higher and serum CRP was lower after rosuvastatin treatment. An additive effect of exercise training was also observed, such that, after the training period, CRP was further lowered and the size of the inflammatory monocyte population in circulation was lower.

The observed increase in TLR4 expression after rosuvastatin treatment in the present study is a novel finding that may explain previous reports of statin treatment augmenting LPS-stimulated inflammatory cytokine production in monocyte/macrophage cells [5-7]. For example, lovastatin treatment of cultured macrophage increased LPS-induced TNF-α production [6]. This effect was abolished with the addition of the cholesterol precursor mevalonate, suggesting that inhibition of cholesterol synthesis or downstream products such as isoprenoids may be involved. Furthermore, treatment of peritoneal macrophage from C57/BL mice with simvastatin potently augmented LPS-induced IL-12p40 and TNF-α production [5]. In addition, statin pretreatment of murine macrophage potentiated proinflammatory cytokine production stimulated by a panel of TLR2 and TLR4 ligands [8].

In contrast to those findings, others have reported that statins suppress the inflammatory response of monocyte/macrophage cells. Statins attenuated LPS activation of cultured monocytes [9] and reduced LPS activation of nuclear factor–κB [10,11]. In patients with hypercholesterolemia, simvastatin inhibited monocyte expression of proinflammatory cytokines TNF-α, IL-1β, and IL-8 [12,13]. With respect to TLR4 expression, Niessner et al [14] reported that simvastatin (80 mg/d) treatment for 4 days did not alter TLR4 or TLR2 cell surface expression on CD14+ monocytes obtained from healthy control subjects [14]. Furthermore, 4 weeks of atorvastatin (20 mg/d) reduced TLR4 expression on CD14+ monocytes from subjects with normal cholesterol levels [15]. It is difficult to reconcile these divergent reports on the basis of statin treatments effects on monocyte/macrophage inflammatory response and TLR4 expression; however, differences in experimental design and treatment duration may play a role. While our observation of increased TLR4 expression after rosuvastatin treatment is congruent with reports of statin treatment augmenting LPS-stimulated inflammatory cytokine production in monocyte/macrophage cells [5-7], more studies are

needed to clarify the role of statin treatment on TLR4-mediated monocyte/macrophage inflammatory response.

In previous studies, we have demonstrated that exercise training reduced monocyte expression of TLR4 in conjunction with a decreased LPS-stimulated cytokine production [23,29]. However, in the present study, there was no change in TLR4 expression in the RE group after the exercise training intervention. Therefore, we hypothesize that the rosuvastatin-induced effect on TLR4 expression prevented the expected exercise training–induced decline [23]. Although this is an attractive interpretation of our data, the lack of an exercise only control group makes it a difficult one to support. The addition of an exercise control group would have possibly allowed us to address this and is a limitation of the study design. The effect of rosuvastatin on TLR4 expression could be interpreted as a negative response when examined within the context of inflammation influencing chronic disease. However, an alternative view is that this response should not necessarily be construed as negative. Toll-like receptors respond to an array of pathogen-associated molecular patterns and are critical players in the innate and adaptive immune responses. Therefore, excessive down-regulation of TLR4 could have as far-reaching negative impact as excessive up-regulation; and further investigation is needed to determine whether this is a favorable response.

When the data from treatment groups were combined (R, RE, and AC), we found that inflammatory monocytes expressed 2 times more TLR4 when compared with classic monocytes. A similar finding was reported by Skinner et al [30], who found that inflammatory monocytes (CD14+ CD16+) expressed 2.5 times greater TLR4 in comparison with classic monocytes (CD14+CD16−). This finding underscores the inflammatory potential of this monocyte subpopulation. Despite constituting a small percentage of the total monocyte population, inflammatory monocytes have a greater proclivity for production of inflammatory cytokines when activated and do not express the anti-inflammatory cytokine IL-10 [31]. For example, Belge et al [32] found that reducing the inflammatory monocyte population significantly impaired the ability of monocyte cultures to produce TNF-α in vitro. Belge et al also found that inflammatory monocytes produced 3-fold more TNF-α than classic monocytes after LPS treatment. Thus, our data support the consensus that CD14+CD16+ monocytes have a greater inflammatory potential. We also observed that exercise training reduced the proportion of circulating inflammatory monocytes. This is consistent with the findings from a recent report wherein a training program decreased inflammatory monocyte percentage in older previously sedentary subjects [19]. These findings may have important clinical implications, as the inflammatory monocyte population seems to be involved in the pathophysiology of numerous diseases. The inflammatory monocyte population is expanded in rheumatoid arthritis patients [33], in cardiovascular disease [34], and also in a NOD mouse model of diabetes [35]. Furthermore, inflammatory monocytes adhere to activated vascular endothelial cells more robustly when compared with classic monocytes and may be antecedent to the CD16+ macrophages found in atherosclerotic lesions [36-38]. Given that inflammatory monocytes play a significant role in the etiology of several inflammatory-related diseases, interventions that lower the population of inflammatory monocytes, such as exercise training, may prove valuable for treatment of these chronic diseases [39].

When data from the R and RE groups were combined, it was found that rosuvastatin treatment reduced serum CRP at 10 and 20 weeks. This result is similar to previous reports describing rosuvastatin-induced decrease in CRP [40] and, given the relatively low baseline CRP of the intervention subjects (~2 mg/L), underscores the ability of statins to reduce a clinical marker of systemic inflammation. Significantly, we show that the addition of exercise training to rosuvastatin treatment resulted in a further decrease in serum CRP. Although reports show that rosuvastatin and exercise independently reduce CRP, this is the first study to describe an additive effect when exercise and statin treatment are combined. This novel finding is clinically relevant because increased physical activity is often prescribed along with rosuvastatin treatment for hypercholesterolemia.

A potential limitation of the present study was that a combination of men and postmenopausal women was used as subjects. Differences in the hormone milieu between sexes and administration of hormone replacement therapy have been shown to affect blood-borne mediators of inflammation [41,42]. However, we observed no sex differences in monocyte TLR4 expression, inflammatory monocyte population size, or serum CRP. Furthermore, although this study included 5 postmenopausal women receiving hormone replacement therapy (AC, n = 2; R, n = 1; RE, n = 2), no effect of hormone replacement therapy on inflammatory measures was observed.

In conclusion, our study provides novel and clinically relevant data describing the effects of rosuvastatin and exercise on mediators of inflammation. These data suggest that rosuvastatin may influence monocyte/macrophage inflammatory response by increasing TLR4 expression on circulating monocytes. The addition of exercise training to rosuvastatin treatment further lowers CRP, a clinical marker of inflammation. Furthermore, exercise training reduced the proportion of the inflammatory monocyte population, a finding that has important implications because this monocyte subpopulation is involved in the pathophysiology of atherosclerosis.

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References

[1] Maron DJ, Fazio S, Linton MF. Current perspectives on statins. Circulation 2000;101:207-13.
[2] Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001; 344:1959-65.
[3] Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. N Engl J Med 1995;333:1301-7.
[4] Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 1996;335: 1001-9.
[5] Matsumoto M, Einhaus D, Gold ES, Aderem A. Simvastatin augments lipopolysaccharide-induced proinflammatory responses in macrophages by differential regulation of the c-Fos and c-Jun transcription factors. J Immunol 2004;172:7377-84.
[6] Monick MM, Powers LS, Butler NS, Hunninghake GW. Inhibition of Rho family GTPases results in increased TNF-alpha production after lipopolysaccharide exposure. J Immunol 2003;171:2625-30.
[7] Konat GW, Krasowska-Zoladek A, Kraszpulski M. Statins enhance toll-like receptor 4–mediated cytokine gene expression in astrocytes: implication of Rho proteins in negative feedback regulation. J Neurosci Res 2008;86:603-9.
[8] Helmers AJ, Gowda DC, Kain KC, Liles WC. Statins fail to improve outcome in experimental cerebral malaria and potentiate Toll-like receptor–mediated cytokine production by murine macrophages. Am J Trop Med Hyg 2009;81:631-7.
[9] Patel TR, Corbett SA. Simvastatin suppresses LPS-induced Akt phosphorylation in the human monocyte cell line THP-1. J Surg Res 2004;116:116-20.
[10] Hodgkinson CP, Ye S. Statins inhibit toll-like receptor 4–mediated lipopolysaccharide signaling and cytokine expression. Pharmacogenet Genomics 2008;18:803-13.
[11] Hilgendorff A, Muth H, Parviz B, Staubitz A, Haberbosch W, Tillmanns H, et al. Statins differ in their ability to block NF-kappaB activation in human blood monocytes. Int J Clin Pharmacol Ther 2003; 41:397-401.
[12] Ferro D, Parrotto S, Basili S, Alessandri C, Violi F. Simvastatin inhibits the monocyte expression of proinflammatory cytokines in patients with hypercholesterolemia. J Am Coll Cardiol 2000;36: 427-31.
[13] Rezaie-Majd A, Maca T, Bucek RA, Valent P, Muller MR, Husslein P, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein–1 in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2002;22:1194-9.
[14] Niessner A, Steiner S, Speidl WS, Pleiner J, Seidinger D, Maurer G, et al. Simvastatin suppresses endotoxin-induced upregulation of toll-like receptors 4 and 2 in vivo. Atherosclerosis 2006;189:408-13.
[15] Methe H, Kim JO, Kofler S, Nabauer M, Weis M. Statins decrease toll-like receptor 4 expression and downstream signaling in human CD14+ monocytes. Arterioscler Thromb Vasc Biol 2005;25:1439-45.
[16] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112:1821-30.
[17] Hansson GK. Inflammatory mechanisms in atherosclerosis. J Thromb Haemost 2009;7(Suppl 1):328-31.
[18] Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Timmerman KL, et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. Med Sci Sports Exerc 2007;39:1714-9.
[19] Timmerman KL, Flynn MG, Coen PM, Markofski MM, Pence BD. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? J Leukocyte biology 2008.
[20] Flynn MG, McFarlin BK, Phillips MD, Stewart LK, Timmerman KL. Toll-like receptor 4 and CD14 mRNA expression are lower in resistive exercise-trained elderly women. J Appl Physiol 2003;95:1833-42.
[21] Smith JK, Dykes R, Douglas JE, Krishnaswamy G, Berk S. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. Jama 1999;281:1722-7.
[22] McFarlin BK, Flynn MG, Campbell WW, Stewart LK, Timmerman KL. TLR4 is lower in resistance-trained older women and related to inflammatory cytokines. Med Sci Sports Exerc 2004;36:1876-83.
[23] Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, McFarlin BK, et al. Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4. Brain Behav Immun 2005;19:389-97.
[24] Troseid M, Lappegard KT, Claudi T, Damas JK, Morkrid L, Brendberg R, et al. Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. Eur Heart J 2004;25: 349-55.
[25] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
[26] ACSM, Kenney WL, Humphrey RH, Bryant CX, Mahler DA. ACSM's guidelines for exercise testing and prescription. 5th ed. Baltimore (Md): Williams and Wilkins; 1995.
[27] Paffenbarger Jr RS, Blair SN, Lee IM, Hyde RT. Measurement of physical activity to assess health effects in free-living populations. Med Sci Sports Exerc 1993;25:60-70.
[28] NCEP. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
[29] McFarlin BK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Stewart LK, et al. Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. J Gerontol A Biol Sci Med Sci 2006;61:388-93.
[30] Skinner NA, MacIsaac CM, Hamilton JA, Visvanathan K. Regulation of toll-like receptor (TLR)2 and TLR4 on CD14dimCD16+ monocytes in response to sepsis-related antigens. Clin Exp Immunol 2005;141: 270-8.
[31] Frankenberger M, Sternsdorf T, Pechumer H, Pforte A, Ziegler-Heitbrock HW. Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis. Blood 1996;87:373-7.
[32] Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, et al. The proinflammatory CD14+CD16+DR+ monocytes are a major source of TNF. J Immunol 2002;168:3536-42.
[33] Baeten D, Boots AM, Steenbakkers PG, Elewaut D, Bos E, Verheijden GF, et al. Human cartilage gp-39+,CD16+ monocytes in peripheral blood and synovium: correlation with joint destruction in rheumatoid arthritis. Arthritis Rheum 2000;43:1233-43.
[34] Schlitt A, Heine GH, Blankenberg S, Espinola-Klein C, Dopheide JF, Bickel C, et al. CD14+CD16+ monocytes in coronary artery disease and their relationship to serum TNF-alpha levels. Thromb Haemost 2004;92:419-24.
[35] Nikolic T, Bouma G, Drexhage HA, Leenen PJ. Diabetes-prone NOD mice show an expanded subpopulation of mature circulating monocytes, which preferentially develop into macrophage-like cells in vitro. J Leukoc Biol 2005;78:70-9.

[36] Ancuta P, Rao R, Moses A, Mehle A, Shaw SK, Luscinskas FW, et al. Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. J Exp Med 2003;197:1701-7.
[37] Hakkinen T, Karkola K, Yla-Herttuala S. Macrophages, smooth muscle cells, endothelial cells, and T-cells express CD40 and CD40L in fatty streaks and more advanced human atherosclerotic lesions. Colocalization with epitopes of oxidized low-density lipoprotein, scavenger receptor, and CD16 (Fc gammaRIII). Virchows Arch 2000; 437:396-405.
[38] Randolph GJ, Sanchez-Schmitz G, Liebman RM, Schakel K. The CD16(+) (FcgammaRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. J Exp Med 2002;196:517-27.
[39] Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-74.
[40] Ferdinand KC, Clark LT, Watson KE, Neal RC, Brown CD, Kong BW, et al. Comparison of efficacy and safety of rosuvastatin versus atorvastatin in African-American patients in a six-week trial. Am J Cardiol 2006;97:229-35.
[41] Rogers A, Eastell R. The effect of 17beta-estradiol on production of cytokines in cultures of peripheral blood. Bone 2001;29:30-4.
[42] Schuurs AH, Verheul HA. Effects of gender and sex steroids on the immune response. J Steroid Biochem 1990;35:157-72.