Effects of Two Weeks of High-intensity Interval Training (HIIT) on Monocyte TLR2 and TLR4 Expression in High BMI Sedentary Men

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ABSTRACT

International Journal of Exercise Science 6(1) : 81-90, 2013. Monocyte TLR expression has been shown to be reduced after a combination of aerobic and resistance exercise, but more studies considering the influences of different exercise intensities, type and duration on TLR expression are needed. Although there is an agreement about the importance of physical exercise, the minimal amount needed to improve health status is uncertain. Therefore, the aim of this study was to analyse the influence of 2 weeks of high-intensity intermittent exercise training on $CD14^+$ monocyte TLR4 expression in a sedentary, high BMI population. As a secondary purpose, this study covers the influence of exercise on classical and pro-inflammatory monocytes and the TLR4 expression before and after a training period in these monocyte subsets. Six high-intensity interval training (HIIT) sessions over a 2 week period (three sessions per week) were completed by 11 sedentary participants (24 ± 5 years old). Blood samples were taken at the beginning and end of the training period for analysis of haematocrit, haemoglobin, total white blood cell (leukocyte), monocyte counts, monocyte $CD14^+$ TLR4 expression and monocyte subsets. Two weeks of high-intensity intermittent exercise training increased VO_{2peak} and total $CD14^+$ monocyte TLR4 expression in a sedentary, high BMI population. There was no influence of training on the proportions of classical and pro-inflammatory monocyte subsets, but TLR4 expression in the majority of these monocyte subsets (apart from $CD14^{++}CD16^+$) was higher after the six training sessions.

KEY WORDS: High intensity interval iraining, Toll-Like receptors; monocyte subsets

INTRODUCTION

It has been suggested that chronic elevation of inflammatory biomarkers is related to chronic disease conditions such as cardiovascular disease (CVD) and diabetes independent of body weight (7, 16). Several researchers have suggested that exercise-induced reduction of toll-like receptors (TLRs) expression and shifts in monocyte phenotype could explain the fall in the inflammatory markers measured (5, 9, 22, 24).

Monocytes are one of the main antigen presenting cells (APCs) found in the circulatory system and the activation of these cells leads to inflammatory responses. According to the amount of cluster of differentiation (CD)14 and CD16 receptors on its surface, three subpopulations of monocyte can be defined: $CD14^{++}CD16^-$

cells (also known as classical monocytes); and more mature subpopulations consisted of $CD14^{++}CD16^+$ cells and $CD14^+CD16^+$ (known as pro-inflammatory monocytes). The classical monocytes consist of around 80-90% of the total circulatory monocyte population, while together both pro-inflammatory populations consist of only 10-20% (19, 25). TLRs are trans-membrane proteins located in APCs, available to recognise an array of pathogen-associated molecular patterns and activate immune responses via intracellular signalling (1, 18). In addition to the recognition role, TLRs are associated with the increased release of cytokines and the stimulation of antimicrobial activity by both the innate and acquired immune system. TLR2 and TLR4 activation stimulates a range of intracellular signalling pathways that coordinate the extent, form and duration of the inflammatory response (for a comprehensive review in TLRs see 3).

Aerobic, long duration exercise at moderate intensity (around 60% VO_{2peak}) results in improved aerobic capacity, increased fat oxidation and increased mitochondrial volume, augmenting the capacity to oxidise fat (4, 11, 12). Nevertheless, studies have shown that HIIT (high intensity interval training = high power output for a few minutes followed by a resting period) results in similar, if not better augmentation of aerobic capacity and fat oxidation even when total exercise time does not exceed 30 min per session (6, 10,11, 19). For example, Talanian et al. (23) demonstrated that 10 repetitions of 4 min at 90% VO_{2peak} followed by 2 min rest increases aerobic capacity, power output, fat oxidation during exercise and skeletal muscle citrate synthase activity in recreationally active women after a two week training period. These findings

suggest that high-intensity intermittent exercise is a powerful training strategy to stimulate adaptations in the skeletal muscle and to improve health in trained and untrained individuals (11, 20, 23).

Stewart et al. (22) demonstrated that 12 weeks of combined aerobic and resistance exercise (3 times per week) reduces TLR4 expression in both the old and young (previous inactive) population with also changes in BMI. Similar findings were shown by Timmerman et al. (24), where 12 weeks of resistance and aerobic training (3 times per week, around 50 min in total) did not change total $CD14^+$ monocyte TLR4 expression but reduced TLR4 expression in pro-inflammatory monocytes ($CD14^+CD16^+$), the pro-inflammatory monocyte.

Monocyte TLR expression has therefore shown to be reduced after a combination of aerobic and resistance exercise, but more studies considering the influences of different exercise intensities, type and duration on TLR expression are needed. As HIIT lasting up to one hour has been shown to result in greater physiological adaptations than moderate exercise of the same duration, it would be beneficial to know if the high-intensity training protocol can also elicit more positive immunological changes. Therefore, the aim of this study was to analyse the influence of 2 weeks of high-intensity intermittent exercise training on $CD14^+$ monocyte TLR4 expression in a sedentary, high BMI population. As a secondary purpose, this study analysed the influence of exercise on classical and pro-inflammatory monocytes and the TLR4 expression before and after a training period in these monocyte subsets.

METHODS

Participants
A total of 11 male participants aged 24 ± 5 years old were recruited through word of mouth, emails and advertisements around the Loughborough University area. Participants were all non-smokers, were not taking any medication and had remained free of symptoms of respiratory infection prior to participation in the study, and only sedentary (no more than 2 exercise sessions per week) males were recruited. All participants had a BMI greater than 25 kg/m2 but were otherwise healthy. Participants' anthropometric and fitness characteristics are shown in Table 1.

This study was part of a larger study conducted in the School of Sport, Exercise and Health Sciences at Loughborough University (13). The volunteers gave informed written and verbal consent after being advised of all possible risks and discomforts associated with the procedures used in the study, and all procedures were submitted to and approved by Loughborough University Ethical Advisory Committee.

Protocol
VO₂ Peak Determination: A VO_{2peak} test was used to determine participants' maximum aerobic capacity at the beginning and end of the study. In brief, participants cycled to volitional exhaustion using a continuous incremental protocol on an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). Expired air was measured continuously for oxygen uptake using an online breath-by-breath gas analysis system (Ultima CPX, Medical Graphics, MN, USA).

VO_{2peak} was identified as the highest VO_2 over a 30-s period during the test.

Familiarisation: After 7 days, participants attended the laboratory to complete a familiarization of the HIIT. During this visit, participants completed six 4-min bouts of cycling with 2-min of rest between intervals. The workload was manipulated during the trial to ensure that the average corresponding VO_2 during exercise equated to ~85% VO_{2peak}.

Anthropometric measurements: Waist-to-hip ratio measurements were also taken during the familiarisation visit. Waist circumference was measured as half-way between the iliac crest and the lowest rib, and hip circumference was measured at the widest part of the hips according to the procedures outlined by the World Health Organisation (WHO).

Training Protocol: As training, participants completed six training sessions over a 2 week period (three sessions per week). Sessions occurred at any time of the day according to the availability of the participants. The sessions consisted of participants performing ten, 4-min cycling intervals separated by 2-min rest period at a work rate corresponding to that which elicited ~85% of a participant's VO_{2peak}. Water was consumed *ad libitum* during exercise. Heart rate measurements were made at the last 30 s of each 4-min stage. The work rate was adjusted (during training sessions if participants had a lower heart rate value when compared with the values achieved in either the main trial or previous sessions.

Blood Sampling and Analysis: Participants arrived in the laboratory at 08:00 after an

overnight fast and rested for 5 min before having their blood sample taking. Blood samples were collected prior to first training session and 48 hours after the last session. 4 ml K₃EDTA and 10 ml heparin vacutainers were collected. Haematocrit, haemoglobin, total white blood cell (leukocyte) and monocyte counts were determined from the K₃EDTA blood using an automated haematology analyser. Heparinised blood was used to analyse monocyte and TLR expression as described below.

Monocyte Subpopulation Determination and TLR Expression: For this study a peripheral blood mononuclear cell (PBMC) method was applied before staining the cells. In brief, 4 ml of Histopaque 1077 (Sigma Aldrich, Dorset, UK) was added into a tube and 4 ml of heparinised blood was then carefully layered on top. The tubes were centrifuged at 400 g for 30 min at 25°C. PBMC layer was then aspirated and transferred to another tube containing Hank's buffered salt solution (HBSS, 1:1 dilution) and centrifuged at 300 g for 10 minutes at 25°C. After that the supernatant was aspirated and cell pellets were re-suspended in phosphate buffered saline (PBS) containing 0.1% of bovine serum albumin (BSA) and 2 mM EDTA.

In addition to the TLR2 and TLR4 expression on classical CD14⁺ monocytes, this study also analysed the CD16⁺ monocyte subsets (also called pro-inflammatory monocytes) response after exercise. For this, the monoclonal antibody CD16 was included in the staining process. Cell staining procedures were similar to those described in Oliveira & Gleeson 2010. In brief, 0.5 ml of PBMC were surface stained with 20 μl CD14-FITC, 20 μl of CD16-PE-Cy5, and antihuman PE-conjugated TLR2 (20 μl), PE-TLR4 (20 μl) or PE-Isotype control (20 μl). All the tubes were incubated at room temperature for 20 min in the dark. In order to lyse the remaining red blood cells, tubes were then filled with a FACS lysing buffer solution (BD Biosciences, Oxford, UK) and re-incubated for 10 min in the same conditions. All samples were then centrifuged at 1000 g for 6 min. Supernatants were aspirated and cell pellets were re-suspended in 500 μl of PBS/BSA/EDTA and transferred to FACS tubes for analysis. Samples were analysed on a flow cytometer equipped with the CellQuest software package. Cells were gated according to side scatter and CD14-FITC expression (which characterises monocytes), and the geometric mean fluorescence intensity (GMFI) of the Isotype
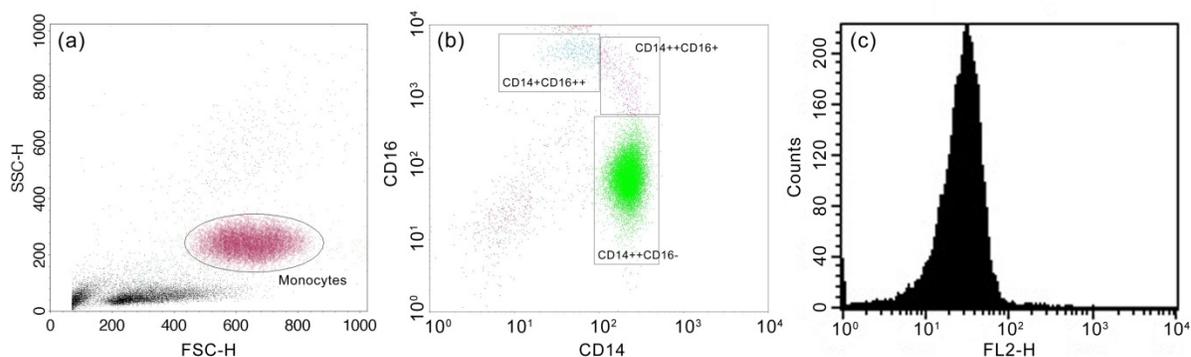

Figure 1. Example of (a) monocyte gating using PBMC method, (b) monocyte subset gating and (c) representative flow cytometry histogram of TLR4 expression.

control (ISO), and of TLR2 and TLR4 antibodies in the CD14$^+$ cell gated population was obtained to quantify TLR expression (total of 20,000 cells acquired for each sample). Isotype control GMFI was used to correct values for non-specific binding. Additional gates were then used to identify the CD14^{++}CD16$^-$, CD14$^+$CD16^{++} and CD14^{++}CD16$^+$ monocyte populations and their co-expression with TLR2 and TLR4. The expression of TLR2 and TLR4 on all CD14$^+$ monocytes was also analysed (Figure 1).

Statistical Analysis
Student's t-test was used to compare before and after training samples in all data. Variables that were not normally distributed (CD14$^+$ and CD14^{++}CD16$^-$ TLR4 expression after training, CD14$^+$ and CD14^{++}CD16$^-$ TLR2 expression before training and CD14$^+$CD16^{++} TLR2 expression) were also analysed using the Wilcoxon signed rank test. The results of the non-parametric test showed similar results to t-tests, and so only t-test values will be presented in the results section. Pearson correlation was used to assess relationship between VO$_{2peak}$, BMI waist-to-hip ratio and TLR expression. In order to calculate these statistics, PASW version 18.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used. Statistical significance was accepted at *P* < 0.05. All results are presented as mean ± standard deviation (SD).

RESULTS

Physiological Characteristics: Participants performed the main high-intensity intermittent training at ~84% of their VO$_{2peak}$ both before and after the 2-week training programme. VO$_{2peak}$ was increased

by 7.6% (*P* = 0.05, Table 1). Percentage of heart rate during exercise was 5% lower after the training programme (*P* = 0.001).

Table 1. Participants characteristics before and after the 2-week training programme.

	Before training	After training	*P* value
Body Mass (kg)	90.0 ± 7.5	89.9 ± 7.6	0.91
Height (m)	1.77 ± 0.05	1.77 ± 0.05	-
BMI (kg·m^{-2})	28.9 ± 3.2	28.9 ± 3.3	0.96
Waist circumference (cm)	96.3 ± 8.4	95.0 ± 8.8	0.06
Hip circumference (cm)	109.4 ± 5.3	108.2 ± 5.8	0.08
Waist-to-hip ratio	0.88 ± 0.05	0.88 ± 0.05	0.46
O$_{2peak}$ (l·min^{-1})	3.40 ± 0.6	3.66 ± 0.5*	0.05
% Heart Rate (bpm)	89.3 ± 2.5	85.0 ± 2.8	0.001

Mean ± SD, BMI: Body Mass Index; * Statistical difference found compared with after.

Blood Cell Count: Participants presented lower red blood cell count and haemoglobin concentration post-training compared with pre-training (*P* < 0.05), but all other variables were unchanged (Table 2).

Monocyte Subpopulation Determination: No significant differences were found in the proportions of the monocyte subpopulations after the exercise training programme (*P* > 0.05), as seen in Table 3.

Monocyte TLR Expression Results: TLR4 expression on CD14 total , CD14^{++}/CD16$^-$, CD14^{++}/CD16$^+$ and CD14$^+$/CD16^{++}

Table 2. Total blood cell count before and after two weeks of training.

	Before training	After training	P value
RBC	4.9 ± 0.7	4.3 ± 0.7*	0.04
HCT (%)	42.6 ± 5.6	39.7 ± 5.9	0.13
HB (g/dL)	15.8 ± 0.9	15.1 ± 1.0*	0.03
WBC (x10^9/L)	5.7 ± 1.4	6.0 ± 2.0	0.56
NEU (x10^9/L)	2.6 ± 0.8	3.2 ± 1.2	0.06
LYM (x10^9/L)	2.2 ± 0.6	2.1 ± 0.7	0.48
MON (x10^9/L)	0.6 ± 0.2	0.6 ± 0.2	1.00
EOS (x10^9/L)	0.2 ± 0.1	0.2 ± 0.1	0.48
BAS (x10^9/L)	0.0 ± 0.1	0.1 ± 0.1	0.34

(RBC = Red blood cells; HCT = Haematocrit; HB = Haemoglobin; WBC = white blood cell; NEU = neutrophil; LYM = Lymphocyte; MON = Monocyte; EOS = Eosinophil; BAS = Basophil); N= 11. * Statistical difference compared with after.

monocyte populations are shown in Figure 2. TLR4 expression was two-fold higher after training on total CD14$^+$ monocytes (t(10)= -2.90, P = 0.02), 2.6-fold higher on CD14^{++}/CD16$^-$ (classical monocyte; t(10)= -3.05, P = 0.01) and 1.5-fold higher on CD14$^+$/CD16^{++} (pro-inflammatory monocyte; t(10)= -2.40, P = 0.04). There was no significant difference in TLR2 expression before and after the training programme (P = 0.21 to 0.48). The GMFI for TLR2 is presented in Table 4. There was no significant correlation between TLR expression and VO$_{2peak}$, BMI and waist-to-hip ratio (all P > 0.05).

Table 3. Monocyte subpopulation before and after two weeks of training programme.

	Before training	After training
CD14^{++}CD16$^-$ (%)	77.0 ± 7.8	74.0 ± 9.2
CD14^{++}CD16$^+$ (%)	4.8 ± 1.8	5.3 ± 2.1
CD14$^+$CD16^{++} (%)	3.9 ± 2.9	4.9 ± 2.5
Non-gated Monocytes (%)	14.3 ± 7.8	14.8 ± 7.5

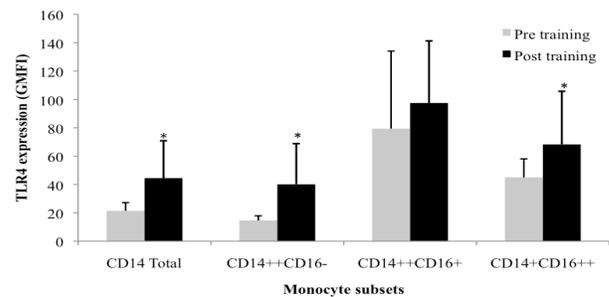No statistical differences (P > 0.05).

Figure 2. TLR4 expression in monocytes before and after the training programme. * Significantly different from pre training (P < 0.05).

Table 4. TLR2 expression in monocytes before and after the training programme.

	CD14 Total	CD14^{++} CD16$^-$	CD14^{++} CD16$^+$	CD14$^+$ CD16^{++}
Pre training (GMFI)	24.7 ± 5.8	14.6 ± 7.2	75.5 ± 30.2	39.7 ± 23.4
Post training (GMFI)	22.4 ± 3.8	16.3 ± 6.7	70.5 ± 24.3	36.9 ± 19.9

DISCUSSION

The aim of this study was to analyse the influence of two weeks of high-intensity intermittent exercise training on CD14$^+$ monocyte TLR2 and TLR4 expression in a sedentary, high BMI population. In addition, as a secondary purpose, this

study examined the influence of exercise on classical and pro-inflammatory monocytes and the effect of the 2-week exercise training period on TLR2 and TLR4 expression in these monocyte subsets. The novel finding from the present study was that six sessions of high-intensity intermittent exercise increased total monocyte TLR4 expression with no changes in the monocyte subset population percentages. This increase in TLR4 expression was also seen in all monocyte subsets apart from CD14^{++}CD16^{+}. In contrast, TLR2 expression was unchanged following exercise training in all monocytes subsets.

To the author's knowledge, this was the first study to analyse the influence of a short period of high-intensity intermittent exercise training on TLR4 expression in a young, high BMI sedentary population. Several previous studies have examined the influence of a combination of resistance and aerobic training on monocyte TLR4 expression (5, 9, 15, 22 24), but the majority of the studies were conducted on elderly women (5, 9, 15, 24). In contrast to the findings of the present study, Timmerman et al. (24) found no difference in TLR4 expression on inflammatory monocytes, classical monocytes or total monocytes after 12 weeks of training in sedentary elderly females (N=15). However, they showed a 64% reduction in the circulating inflammatory monocyte numbers after 12 weeks of training. Reasons for such a difference in the findings may be attributed to differences in sex, age and physical activity status of the participants between studies. Another reason for differences between the present study and other training studies cited previously was that the exercise protocol, including the

intensity and type of exercise, was different, although the total duration of the each session was approximately the same (~ 50 to 60 min).

In the present study training did not significantly change total body weight, BMI or waist-to-hip circumference. It can therefore be suggested that the changes observed in the monocyte TLR4 expression may occur irrespective of BMI. So far, no studies have been performed on TLR4 expression in young high BMI sedentary males and so no direct comparisons can be made. However, changes in TLR4 expression (either increases or decreases) have been shown to be independent of BMI values in other training studies (22, 24).

Although the high-intensity exercise training protocol increased VO$_{2peak}$ by 7.6%, the increase in the monocyte TLR4 expression that occurred may not be a positive outcome for a sedentary population. High TLR4 expression may be linked to low-grade chronic inflammation, which is linked to the occurrence of diseases. Booth et al. (2) reported increased monocyte TLR4 expression immediately and 1 h after both male and female well-trained participants performed a 60 km indoor cycling time trial; however, no follow up was performed to see if these values were higher a few hours after the exercise. In contrast, it has been observed that monocyte TLR4 expression falls but then returns to baseline levels 4 h after long duration exercise (17). Therefore, the changes in the monocyte TLR4 expression seen after a single bout of exercise may not reflect similar findings as a training study. The results found in the present study showed an increased TLR4 expression compared with the beginning of the

training programme even though blood samples were taken 48 hours after the end of the last exercise training session.

Nevertheless, acute inflammatory responses may be one of the stimuli that provoke training adaptations in exercised muscle, and people who exercise more have lower circulating levels of inflammatory markers (16). These support the idea that exercise has anti-inflammatory effects. The rationale for this may be due to the cross-tolerance or desensitisation that physical training may exert on some immune cells such as monocytes (8). Even though the present study showed an increase in the total monocyte CD14$^+$ TLR4 expression and in the majority of the monocyte subset TLR4 expression, this may be a transitory effect until homeostasis reoccurs in the system. These results may be because participants were not used to any kind of physical exercise, and were even less used to high-intensity efforts. Nonetheless, it is worth noting that the high-intensity training protocol resulted in no difference in plasma IL-6 and TNF-α concentration (13). Smart et al. (21) meta-analysed the effects of different training protocols on IL-6 and TNF- α in heart failure patients. They found that although exercise training increases VO$_{2peak}$ by around 8% this is not correlated to the reduced levels of TNF- α caused by exercise. In addition, IL-6 was unchanged. It can be speculated that even with high TLR4 expression after two weeks of training, other factors may help to reduce, or at least avoid, the increase of cytokine production in a sedentary population. Factors such as changes in the TLR4 pathway activation through augmented endogenous ligands in the blood (e.g. plasma IL-1ra, heat shock proteins) may contribute to that. To support

this idea, a control group and analysis of various cytokine production rates would be necessary. It is important to note that depending on the ligand, TLR4 expression elicits different responses, which will influence the type, magnitude and duration of the inflammatory response. For example, the TLR4/MyD88 signalling pathway is used to induce the expression of pro-inflammatory cytokines, while TLR2/MyD88 stimulates the production of Th2 cells (3). If TLR4 links with TRAM, TRIF and subsequently associates with TRAF3, this pathway will result in the production of IFN-β (which is involved in the innate immune response activation) and the production of the anti-inflammatory cytokine IL-10 (3). Once again, direct comparisons with other studies are difficult to make when studies examining the effects of high-intensity exercise training programmes on TLR4 pathway or cytokine production have not yet been published.

On the other hand, it has been suggested that surgical operations cause variation in the immunological parameters in man (14). With the immunodepression caused by surgery the host defences may be compromised, providing an open window for bacterial infection, making the recovery process harder. It can therefore be suggested that training exercise such as HIIT may be a good aid to boost immune system in times of immunodepression such as during post-operatory periods.

To conclude, two weeks of high-intensity intermittent exercise training increased total CD14$^+$ monocyte TLR4 expression in a sedentary, high BMI population. There was no influence of training on the proportions of classical and pro-inflammatory monocyte subsets, but TLR4 expression in

the majority of these monocyte subsets was higher after the six training sessions. It is not certain that the effects seen in this study were due to the very short (2 weeks) duration of the training; therefore, a considerably longer duration study with the addition of cytokine production analysis may help to establish if the elevation on monocyte TLR4 expression is only a transitory effect, and help to obtain clearer results on this topic.

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