Exercise and Toll-like receptors

Running Head: Exercise and TLRs

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Abstract

Toll-like receptors (TLRs) are highly conserved trans-membrane proteins that play an important role in the detection and recognition of microbial pathogens. The key product of TLR signalling in antigen presenting cells is the production of inflammatory cytokines and proteins. The TLR pathway plays an important role in mediating whole body inflammation, which has been implicated in the development of chronic disease. An accumulation of chronic, low-grade inflammation is common in individuals that live a sedentary lifestyle; however, the mechanism underlying this connection is not fully understood. There is evidence to show that TLRs may be involved in the link between a sedentary lifestyle, inflammation, and disease. Recent studies have shown that both acute aerobic and chronic resistance exercise resulted in decreased monocyte cell-surface expression of TLRs. Furthermore, a period of chronic exercise training decreases both inflammatory cytokine production and the cell-surface expression of TLR4 on monocytes. These effects may contribute to post-exercise immunodepression and the reported higher susceptibility to infection in athletes. However, over the long term, a decrease in TLR expression may represent a beneficial effect because it decreases the inflammatory capacity of leukocytes, thus altering whole body chronic inflammation. The precise physiological stimulus mediating an exercise-induced decrease in cell-surface TLR expression is not known; however, a number of possible signals have been implicated including anti-inflammatory cytokines, stress hormones and heat shock proteins.
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Introduction: The role of Toll-like receptors

Toll receptors are trans-membrane proteins that are highly conserved in animal phyla from insects to mammals, including humans (43, 49). A Toll protein was originally discovered in the fruit fly *(Drosophilia),* and was found to play an important role in ontogenesis and antifungal defence (43). In 1997, Medzhitov et al (49) identified and characterised a human homologue of the *Drosophila* Toll protein and named it a Toll-like receptor (TLR). Thus far 11 human TLRs (TLR1-11) have been identified (83), and they appear to play roles in pathogen detection and recognition and the induction of antimicrobial activity by both the innate and acquired immune system (82-84)

In mammals, TLRs function as pattern recognition receptors that recognise conserved pathogen-associated molecular patterns (PAMPs) expressed by a wide spectrum of infectious microorganisms. TLRs are central in the detection and recognition of pathogen subtypes including gram-positive and gram-negative bacteria, DNA and RNA viruses, fungi and protozoa (52). The specific PAMPs recognised by TLR family members have been well characterised: TLR2 homodimers and TLR2-TLR1 and TLR2-TLR6 heterodimers mediate responses to bacterial lipoproteins, peptidoglycan, lipoteichoic acid and zymosan (57, 73, 87); TLR3 to double-stranded RNA, a marker of viral infection (1); TLR4 to bacterial lipopolysaccharide (LPS) (29, 63); TLR5 to bacterial flagellin (25); TLR7 and 8 to imidazoquinolines and single stranded RNA (27, 34); and TLR9 to bacterial DNA (28). As PAMPs are not expressed by host cells, TLR recognition of PAMPs permits self-nonself discrimination. Nevertheless, there are several endogenous ligands of TLRs that may play a role in regulating the expression of the receptors (37) or allow the immune system to respond to damage or "danger signals."

As illustrated in Figure 1, the recognition of PAMPs by TLRs triggers intracellular signalling pathways (85) and results in induction of a conserved host defence programme which includes the production of inflammatory cytokines (1, 49) and the induction of antimicrobial activity (3), allowing the host to respond immediately to microbial invasion. It is now clear that TLRs on monocytes, macrophages, and dendritic cells contribute significantly to the development of adaptive immune responses (59). Activation of TLRs on these antigen presenting cells (APCs) results in both the activation of innate immune responses by inducing antimicrobial activity (3, 88), the production of inflammatory cytokines including IL-1β, IL-6, IL-8, and TNF-α (8, 48, 58). It also results in the generation of an adaptive immune response through the up-regulation of major histocompatibility complex class II (MHCII) and co-stimulatory molecule expression (CD80/86) (1, 8, 94) on APCs and the release of IL-12 from dendritic cells (DCs). Although the induction of MHCII and CD80/86 is critical to naïve T cell activation, the generation of adaptive immunity is also controlled by $CD4^+CD25^+$ suppressor or regulatory T (T_R) cells (8). A critical role for TLRs in regulating the suppressor activity of T_R cells has recently been reported. Specifically, Pasare & Medzhitov (58) demonstrated that IL-6 production by DCs following TLR activation is essential to the activation of pathogen-specific T cells by inhibiting the suppressive effects of $CD4^+CD25^+$ T_R cells.

Therefore, TLRs, through pathogen recognition and the control of innate and adaptive immune responses, play a pivotal role in the host defence response

against infection. Indeed, the importance of the TLR signalling pathway in mammalian immunity is evident from studies involving rodents with specific TLR deficiencies that have examined the role of the Toll family of receptors and their cognate downstream signalling molecules (2, 52, 70, 72, 86). Furthermore, human studies provide evidence that the expression and activation of TLRs *in vivo* contributes to host defence against microbial pathogens (40) and the effective generation of specific antibodies following vaccination (2).

	22°C	37°C	40°C
TLR1	20.5 ± 3.8	20.5 ± 7.2	18.9 ± 3.1
TLR2	109.5 ± 10.4	121.8 ± 14.3	99.9 ± 11.4
TLR4	33.8 ± 2.7	32.0 ± 4.0	28.8 ± 3.5
CD14	434 ± 15	401 ± 16	424 ± 22

Table 1. The effect of incubation for 1.5 h at room temperature (22°C), 37°C and 40°C on TLR and CD14 expression on CD14+ monocytes. All values are mean ± SEM geometric mean fluorescence intensity (GMFI). Previously unpublished data.

TLRs also interact with a variety of endogenous human ligands and influence the activity of a wide range of tissues and cell processes (35). TLRs are also known to play a role in asthma, coronary heart diseases, inflammatory bowel disease, rheumatoid arthritis and transplant rejection (15). Many important opportunities for disease modification through TLR manipulation can be imagined. The recent findings that both acute and chronic exercise result in lowered expression of some TLRs on APCs offers a new mechanism to explain some of the effects of exercise on immune function as well as the longer term health benefits of a more active lifestyle in reducing the risk of cardiovascular and metabolic diseases which are commonly associated with elevated levels of inflammation markers including cytokines.

Acute exercise and monocyte TLR expression

Only a small number of studies have examined the effect of exercise training on TLR expression (these are described later in this review), and the effect of acute exercise on TLR expression has received even less attention. Lancaster et al (42) were the first to report a decrease in monocyte (CD14+) TLR expression and function following a single bout of prolonged aerobic exercise. Specifically they investigated the influence of 1.5 h of strenuous cycling exercise (~65 % $VO_{2\ max}$) in the heat (34 ºC) on TLR expression and function in vivo. TLR1, TLR2 and TLR4 expression were significantly lower at post-exercise and after 2 h recovery compared to samples obtained at rest. In contrast, TLR9 expression was unaffected by exercise. Lancaster et al (42) exercised subjects in the heat (34 ºC) to enhance the exercise-induced stress response, and maximise the likelihood of observing an effect of exercise on TLR expression. Subjects in this study would have experienced much larger increases in body temperature than if they had exercised under normal conditions. As a result it is unclear whether the observed changes in TLR expression were the result of physiological changes induced by exercise, or physiological changes induced by sizable increases in body temperature comparable to those observed in febrile illness.

To determine if there is a direct effect of temperature on TLR expression, in the Loughborough laboratory we obtained peripheral blood samples from 8

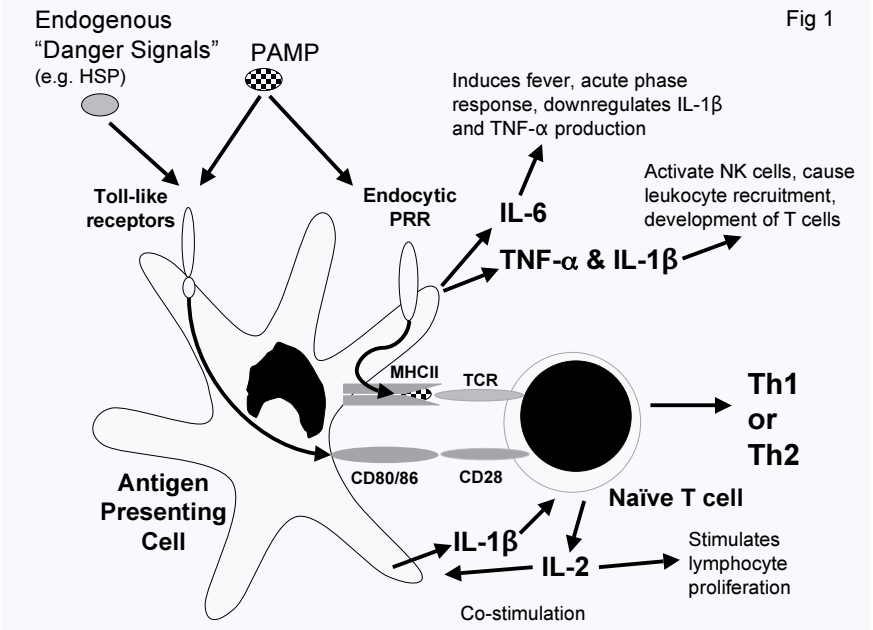

Figure 1: Binding of pathogen associated molecular patterns (PAMPs) and endogenous danger signal molecules such as heat shock proteins (HSP) to Toll-like receptors (TLRs) leads to activation of the antigen presenting cell (APC) and subsequent activation of T-helper (Th) cells that it interacts with. APCs take up antigen via endocytic pattern recognition receptors (PRRs) and process (degrade) it to immunogenic peptides which are displayed to T cell receptors (TCRs) in the polymorphic grove of MHC class II molecules after their appearance at the cell surface. An interaction occurs between the APC and the T cell as indicated, usually resulting in cellular activation. When naive CD4+ T helper (Th) cells are activated by APCs that provide appropriate co-stimulatory signals (cytokines and/or accessory binding molecules), they differentiate into Th1 or Th2 cells with polarised cytokine secretion. Cytokines produced by APCs and Th cells result in inflammation and proliferation and activation of other immune components.

healthy male volunteers (mean ± SD) (age 27 ± 11 yrs) at 09.00. Blood samples from each participant were incubated at room temperature (22 °C), 37 °C and 40 °C for 1.5 h and the expression of TLR1, 2 and 4 and CD14 on the cell surface of monocytes was measured as described by Lancaster et al. (42). Briefly, whole blood was surface stained with CD-14 FITC (Becton Dickinson Biosciences, Oxford, UK) and antihuman PE-conjugated TLR1 (clone GD2.F4), TLR2 (clone TL2.1) or TLR4 (clone HTA 125) antibody (e-Bioscience, San Diego, CA) or the appropriate isotype control for TLR1 (mouse IgG1-PE), TLR2 and TLR4 (both mouse IgG2a-PE). Samples were analyzed on a flow cytometer (BD FACSCalibur) equipped with CellQuest software package (BD Biosciences). Cells were gated according to side-scatter and CD14-FITC expression and the change in the geometric mean fluorescence intensity (GMFI) between TLR1, 2 and 4 antibodies and isotype controls was obtained to quantify TLR expression on CD14+ cells (5000 cells were analyzed). No significant effect of temperature was found for

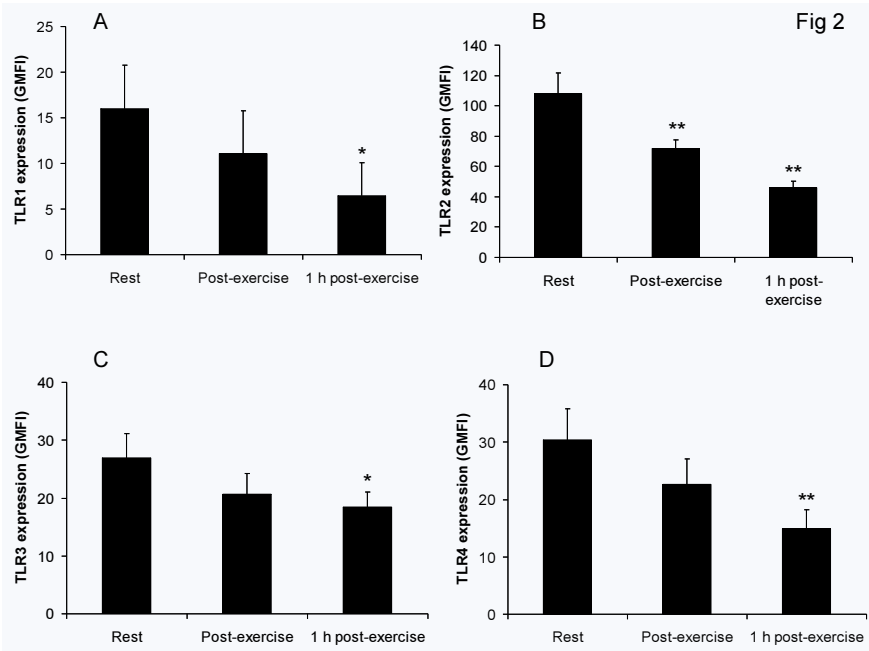

Figure 2: The effect of 2.5 h cycling at 60% VO2max on monocyte (CD14+) cell-surface expression of (A) TLR1, (B) TLR2, (C) TLR3 and (D) TLR4. Significantly less than pre-exercise (Rest) as determined by paired-samples t-test: * P<0.05, ** P<0.01. Data are mean ± SEM geometric mean fluorescence intensity (GMFI) from 11 recreationally active men. Previously unpublished data.

CD14, TLR1, TLR2 or TLR4 expression on CD14+ cells as illustrated in Table 1 Thus, an increase in blood temperature to febrile levels does not appear to alter monocyte TLR expression. These findings are in agreement with Zhou et al (96) who reported that one hour of exposure to 42 ºC followed by a further 6 h incubation at 42 ºC resulted in no change in cell-surface expression of CD14, TLR2 or TLR4 on cultured human monocytes despite an upregulation of mRNA for TLR2 and TLR4 that was associated with elevated cytoplasmic levels of HSP70.

Furthermore, we have recently examined the effects of prolonged exercise in temperate conditions on monocyte cell surface expression of TLRs 1, 2, 3 and 4. Peripheral blood samples were obtained from 11 endurance trained male cyclists (age 20 ± 2 yrs, maximal oxygen uptake ($VO_{2\,max}$) 57.2 ± 8.8 $ml.kg^{-1}.min^{-1}$) at rest and following 2.5 h cycling at 60 % $VO_{2\,max}$ in conditions of 20 ± 2 ºC and 40 ± 5 % relative humidity. Flow cytometry methods were the same as described above; for TLR3 an anti-human PE-conjugated antibody (clone TLR3.7) and mouse IgG1-PE isotype control were used (e-Bioscience). There was a trend for TLR1 expression to be lower following exercise compared with rest (P = 0.07; see Figure 2A) and a significant main effect of time was found for TLR2 (P = 0.004), TLR3 (P = 0.031) and TLR4 (P = 0.003; see Figures 2*B*, *C* and *D*). Core temperature assessed using an oral thermometer was not increased by more than 0.5 ºC in any of the subjects at the end of the exercise bout. Taken

together these findings suggest that acute exercise can result in decreased monocyte TLR expression but this effect is independent of changes in core temperature.

In contrast to these findings, McFarlin et al (47) found no change in monocyte (CD14+) cell surface expression of TLR4 following an acute bout of resistance exercise in trained and untrained elderly women. However, the exercise bout that involved 3 sets of 10 repetitions at 80 % of the 1-repetition maximum for each of 9 different muscle groups lasted only about 1 hour. The exercise used in this study may not have been of sufficient duration to induce a fall in monocyte TLR expression.

Implications for immunity and susceptibility to infection

TLR3 is expressed both intracellularly and on the cell surface. To our knowledge the study described above is the first study to demonstrate that cell-surface TLR3 expression is depressed by exercise. Since TLR3 detects double-stranded RNA—a molecular pattern associated with the presence of viral infection—this could be an important factor in the apparently increased risk of viral infection following very prolonged bouts of strenuous exercise. Although at

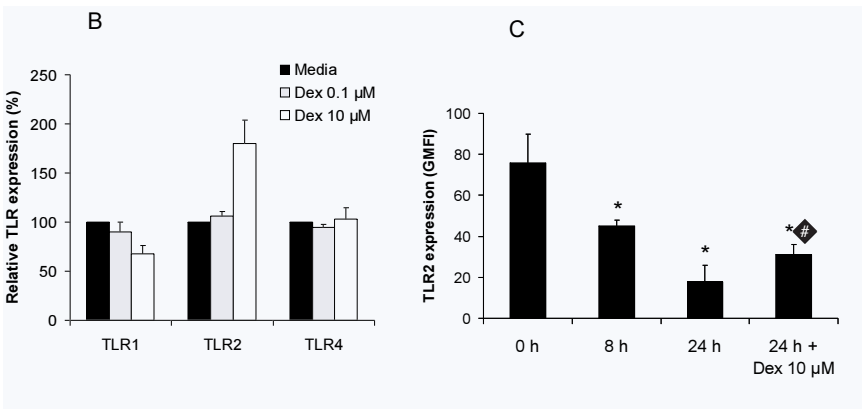

Figure 3: Effect of dexamethasone (Dex) on monocyte (CD14+) cell surface expression of TLR1, 2 and 4. Whole blood was incubated with either media or dexamethasone (10 µM or 0.1 µM) for either (A) 8 h or 24 h (B and C). The data in Figure A represent the mean ± SEM of samples performed in duplicate from 8 individual donors. Significant difference from media only as determined by paired-samples t-test: * $P<0.05$. The data in Figures B and C represent the mean ± SEM from 4 independent experiments performed in duplicate. The values for TLR expression in Figure C are expressed relative to samples incubated with media only. Significant difference from 0 h as determined by paired-samples t-test: * $P<0.05$. Significant difference from 24 h (without Dex) as determined by paired-samples t-test: # $P<0.05$. GMFI: geometric mean fluorescence intensity. Data from Lancaster (41).

present this is speculative there is some evidence that TLR3 is an essential component in innate immune defence against viral infection in mice (81). It is also known that activation of TLR3 induces the activation of NF-kappa-B, the production of type 1 interferons, and inflammatory cytokines (36) which are important mediators of host defence against viral infection. Exercise-induced down-regulation of TLR2 and TLR4 expression has been shown to be associated with reduced MHC II and co-stimulatory molecule (CD80 and CD86) expression and reduced IL-6 secretion by monocytes exposed to TLR2 and 4 ligands (42). These changes might similarly decrease host defence against bacterial infection.

We have used blood monocytes in these studies because it is not possible to collect a sufficient number of tissue macrophages when using a human model. Since monocytes (a less mature form of macrophage) and macrophages share a similar linear progression of their lifespan, it is possible that monocytes are good proxy measures of macrophage activity. As with other published immunological methods, we acknowledge that in vitro measurements may not be reflective of in vivo responses.

Mechanisms for exercise-induced changes in TLR expression

Although at present the mechanisms through which exercise suppresses TLR expression are unknown, altered cell populations, increased levels of circulating cytokines, heat shock proteins (HSPs), and glucocorticoids are obvious candidates. It could be argued that the effects of acute prolonged aerobic exercise on TLR expression could be due to the differential mobilisation of monocyte subsets to the circulation. Two monocyte populations have been identified in human blood, the $CD14^{++}CD16^{-}HLA\text{-}DR^{+}$ classical monocytes and the $CD14^{+}CD16^{+}HLA\text{-}DR^{++}$ pro-inflammatory monocytes (97). The $CD14^{+}CD16^{+}$ pro-inflammatory monocytes have a higher surface expression of TLR2 and produce a greater amount of TNF-α following treatment with TLR2 ligands compared with the $CD14^{+}CD16^{-}$ classical monocytes (7). However, $CD14^{+}CD16^{+}$ monocytes are released from the marginal pool and mobilised into the circulation to a greater extent than $CD14^{+}CD16^{-}$ monocytes during exercise (79) indicating that the effects of prolonged exercise on TLR expression that we have observed are not likely due to the mobilisation of phenotypically distinct monocyte subsets.

TLR activation is known to induce cytokine release (1, 49), but TLR expression appears to be modulated by cytokines. Staege et al (78) reported that in vitro TLR2 and TLR4 expression was down-regulated by interleukin-4 (IL-4) treatment to one-fifth and one-quarter of the level found in untreated cells, respectively. Other authors reported that TLR expression was influenced by cytokine concentrations both in vitro and in vivo (50, 74). It is widely accepted that the circulating concentrations of several cytokines are increased following exercise (56, 90), and it is possible that exercise-induced elevations in cytokines may suppress TLR expression.

Stress hormones, such as glucocorticoids (GCs), mediate many of the immunological changes associated with exercise (61). Lancaster (41) recently investigated the effects of *in vitro* treatment of human blood from 8 healthy donors with the synthetic GC dexamethasone (DEX). Incubation of whole blood for 8 h at 37 ºC (5 % CO_2), at DEX concentrations which resulted in a 50 % (0.1μM) and 100 % (10μM) inhibition of TLR2/TL6 dimer and TLR4 function (induction of IL-6 expression by LPS), resulted in a modest and concentration-dependent decrease in the surface expression of monocyte TLRs 1, 2 and 4 (Figure 3A). While DEX treatment

resulted in a highly reproducible decrease in the surface expression of TLR1, which was observed in all donors, 3 of the 8 donors showed no effect of DEX treatment on either TLR2 or TLR4 expression which could suggest a differential regulation of TLR expression by GCs. In support of this notion, peripheral blood mononuclear cells treated with GCs for 18 h showed a down-regulation of TLR3 gene expression, while TLR2 and TLR4 gene expression was markedly elevated (22). To further examine GC regulation of monocyte TLR expression, Lancaster (41) incubated whole blood with DEX for 24 h. Similar to the results obtained following 8 h of DEX treatment, 24 h of DEX treatment caused a moderate and concentration-dependent decrease in the surface expression of TLR1 (Figure 3B). Furthermore, and in agreement with the study by Galon et al (22), Lancaster (41) observed a significant increase in TLR2 expression in samples treated with 10 μM DEX for 24 h compared with the untreated controls (Figure 3C). However, in contrast to the study by Galon et al (22), there appeared to be no effect of 24 h DEX treatment on monocyte TLR4 expression (41). Galon et al (22) only examined TLR2 and TLR4 gene expression at a single time point. The Lancaster (41) data demonstrate that there is a time-dependent down-regulation of monocyte TLR2 expression that occurs *in vitro* (Figure 3C). Therefore, instead of up-regulating TLR expression *per se*, prolonged DEX treatment appears to attenuate the down-regulation of TLR2 that occurs over time when in culture. Taken together, these results suggest that GCs are able to modulate monocyte TLR expression, and that members of the TLR family are differentially sensitive to GC treatment. However, whether the effects of GCs are mediated directly or indirectly – possibly as a result of a modulation of the release of soluble factors capable of influencing TLR expression, e.g. cytokines – awaits further exploration. In addition, other researchers reporting that GCs altered TLR expression found that GCs induce rather than suppress TLR expression (22, 32). Therefore, it appears unlikely that GCs play a vital role in the down-regulation of TLR expression with exercise. GCs are also known to deplete the inflammatory monocyte population, which could influence TLR expression.

Given the central role of TLRs in innate and adaptive immunity, additional research is needed to identify the mechanisms by which acute exercise regulates and suppresses TLR expression. Another question to which we do not yet have the answer is what happens to the TLRs that disappear from the cell surface during exercise? Are the TLRs shed from the surface or are they internalised? If the latter is true, are they degraded? Other questions include the length of time required for TLR expression to recover following an acute bout of prolonged exercise and the dose of exercise required to induce a decrease in monocyte TLR expression?

Effects of exercise training on monocyte TLR expression

Only a handful of studies have examined the effect of chronic exercise training on TLR expression (20, 80) or compared trained and untrained individuals (46, 47). The results of these studies are summarised in Figures 4, 5 and 6. Flynn et al. (20) initially found that 10 weeks of resistance exercise training significantly lowered LPS-stimulated production of IL-6, IL-1β, and TNF-α (20). In an effort to identify a mechanism that may explain the initial findings, whole blood TLR4 mRNA content was measured. We found that resistance-trained older women (65-85

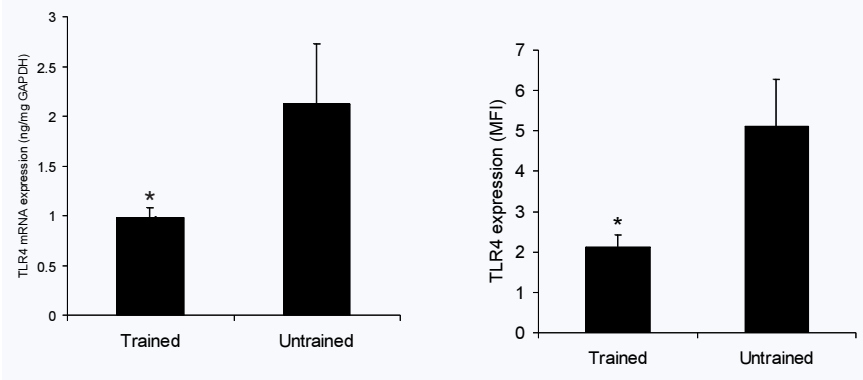

Figure 4: This figure summarizes the key findings with respect to TLR4 expression from the following studies: **A**) Cross-sectional comparison of elderly women following a 9-week resistance training programme (n=9) with untrained controls (n=6) (20); **B**) Cross-sectional comparison of elderly women examining the effects of activity status (n=10 trained and n=10 untrained) (47). * indicates trained significantly lower than untrained ($P<0.05$).

years old) had significantly lower TLR4 mRNA than sedentary untrained older women (20) (Figure 4A). The study design did not allow an analysis of the influence of an acute bout of resistance exercise on TLR expression. Therefore, a follow-up study was conducted in which the effect of a single bout of resistance exercise in trained and untrained older women on CD14+ cell-surface TLR expression (47) was compared. TLR4 expression was measured on CD14+ cells from venous blood prior to exercise, and immediately, 2 h, 6 h, and 24 h after an acute bout of resistance exercise. LPS stimulated whole blood cultures were run in parallel with the flow analysis (20). Exercise training status, but not the acute exercise session, influenced cell-surface TLR4 expression (47) (Figure 4B), such that TLR4 expression in trained subjects was roughly half of the untrained. We also found that high monocyte TLR4 expression was associated with high LPS-stimulated IL-6, TNF-α, and IL-1β production (47).

An early focus on older women (20, 47) prevented Flynn's group from determining whether training effects on TLR4 expression would be present in men and younger individuals. Follow-up studies were designed to address these gaps (46, 80). We recruited participants to fill one of the following groups (46): older, physically active (i.e. trained); older, physically inactive (i.e. untrained); younger, physically active; or younger, physically inactive. Physical activity status, but not age, influenced TLR4 cell-surface expression and LPS-stimulated inflammatory cytokine production (46). Physically active individuals had lower cell-surface TLR4 expression and lower LPS-stimulated inflammatory cytokine production than physically inactive individuals (46) (Figure 5). In a subsequent study, groups of older and younger physically inactive subjects, similar to those in the cross-sectional study, were endurance and resistance exercise trained for 12 weeks (80). Groups of age-, gender-, and health status-matched physically active individuals maintained habitual activity and served as controls. Exercise training (combined aerobic and resistance) significantly decreased cell-surface TLR4

expression (Figure 6) and LPS-stimulated inflammatory cytokine production (80). To our knowledge, these are the only published studies in which the TLR expression following a period of exercise training has been examined. These studies only provide descriptive evaluation of TLR4 and TLR2, making the next logical step to complete an evaluation of mechanisms to determine how exercise training suppresses TLR expression and alters inflammatory cytokine production capacity. Also, more descriptive research is needed to evaluate the effect of chronic exercise training on TLRs other than TLR4 and TLR2.

Implications for long-term health

The accumulation of chronic, low-grade inflammation has been linked to the development of a number of diseases, such as type 2 diabetes mellitus and cardiovascular diseases (13, 30, 77). Based on the literature, whole body chronic inflammation appears to be highest in individuals who are sedentary and/or obese (60). The most effective countermeasures against the accumulation of chronic inflammation appear to be a physically active lifestyle (3-5 days of exercise per week) and maintenance of a healthy body weight (i.e. a body mass index of less than 25 kg/m^2) (60). Physically active participants have significantly lower cell-surface TLR4 expression and monocyte inflammatory cytokine production capacity than physically inactive subjects (20, 46, 47, 80). Blood monocyte TLR cell-surface expression and inflammatory capacity may directly or indirectly affect an individual's level of whole body chronic inflammation. Direct effects are associated with production and release of inflammatory cytokines into the blood, while indirect effects are associated with the ability of monocyte/macrophage-derived inflammatory cytokines to stimulate the release of acute phase proteins from the liver and influence the activity of peripheral tissue macrophages.

In addition to assessing blood monocytes as a direct source of whole body inflammation, they may be a good proxy/convenience measure of the TLR expression and inflammatory capacity of macrophages found in adipose tissue, skeletal muscle, and other peripheral tissue compartments. In adipose tissue, the majority of the TNF-α and about half the IL-6 released comes from adipose tissue macrophages (19, 75). Weight loss has been reported to exert anti-inflammatory affects by decreasing the release of TNF-α from adipose tissue (39, 68) and based on the previous statement, it is clear that this occurs due to changes in macrophage TNF-α production (39, 53). The TLR4 pathway is responsible for

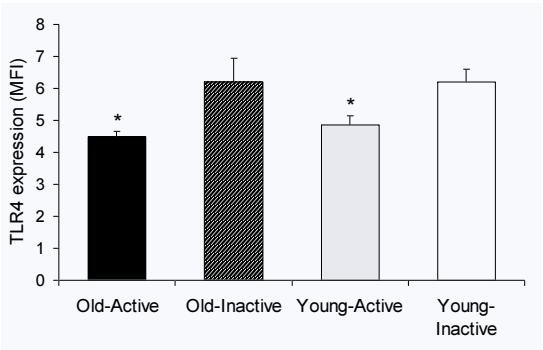

Figure 5: Cell surface (CD14+ cells) TLR4 expression (mean fluorescence intensity, MFI) in old (65-80 years) and young (18-35 years) physically active and inactive subjects (46). Subject numbers in each group were as follows: old, active n= 23; old, inactive n=21; young, active n=21; young, inactive n=19. * indicates active significantly lower than inactive ($P<0.05$).

macrophage production of TNF-α (92), which may be negatively influenced by weight loss. Macrophages from skeletal muscle and other peripheral tissue compartments may be similarly affected; however, to our knowledge this has not been confirmed.

The pathophysiologic link between whole body chronic inflammation and the development of inflammatory-related disease is well documented (13, 30, 77). Monocytes and peripheral tissue macrophages are responsible for an elevation in whole body chronic inflammation. According to the literature, TLR pathways are responsible for mediating the capacity of monocytes and macrophages to produce inflammation (8, 59, 82). More research is needed to address the possible mechanistic links between altered TLR expression and risk of chronic disease and other gaps in the literature.

Ageing, inflammation, exercise, and Toll-like receptors

It is not currently known whether inflammatory dysfunction is the cause or the result of the ageing process (69). Nevertheless, there is a considerable amount of published research to support the contention that ageing is associated with higher levels of inflammatory biomarkers (12, 17, 18, 66) In contrast, Beharka et al. (6) found that inflammatory markers were similar between younger and older subjects when subjects with chronic disease were excluded from the data set. Similarly, Flynn's group found similar LPS-stimulated inflammatory cytokine production and C-reactive protein (CRP) levels in healthy older and younger subjects—provided they were grouped by physical activity level. That is, physically active subjects had significantly lower biomarkers of inflammation, irrespective of age group, than physically inactive subjects. There is a growing consensus that exercise training or high levels of physical activity have anti-inflammatory effects (4, 21, 23, 55, 80, 89, 95). Ford et al. (21) for example, demonstrated a strong independent influence of physical activity on CRP with odds ratio for elevated CRP levels (95 % confidence intervals) of 0.98 (0.78-1.23), 0.85 (0.70-1.02), and 0.53 (0.40-0.71) for those who reported light, moderate, or vigorous physical activity, respectively. Some researchers concluded that body fat changes are responsible for the so-called anti-inflammatory effect of exercise (24, 53, 91), but the weight of evidence appears to be in favour of exercise exerting anti-inflammatory effects in the absence of changes in body fat (23, 55, 80).

Poorly regulated inflammation in the older population is linked to an increase in chronic diseases (11). Toll-like receptors or TLR signaling are linked to chronic diseases such as vascular disease (16, 93) and osteoporosis (33, 44). Nevertheless, there is uncertainty regarding the cause of the observed elevation in inflammatory biomarkers in older adults. It remains to be shown that physical inactivity plays a significant role in the elevated inflammatory status of the elderly cohort.

Ageing and Toll-like receptors

Few researchers have examined age-related differences in toll-like receptor expression. Much of what we currently know comes from animal research (9, 10, 65), but comparisons of human and murine toll-like receptor responses may be complicated

by substantial inter-species differences (26, 64). For example, LPS stimulation increased TLR4 expression in human monocytes and neutrophils (51), but LPS did not increase TLR4 expression in murine macrophages (45).

Boehmer et al (9) observed no effect of age on TLR4 expression in mice but explained an age-related defect in LPS-stimulated cytokine production with evidence of significantly lower mitogen-activated protein kinase (MAPK) signalling (10). Therefore, the TLR4 signaling pathway was defective, but the defect was attributed to MAPK activation and not a result of reduced receptor expression.

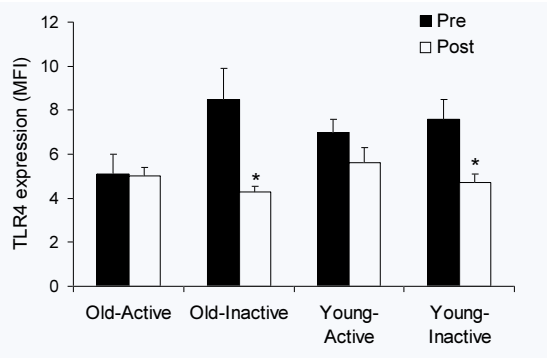

Figure 6: Cell surface (CD14+ cells) TLR4 expression (mean fluorescence intensity, MFI) in old (65-80 years) and young (18-35 years) physically active and inactive subjects before (Pre) and after (Post) 12 weeks of endurance and resistance training for the physically inactive subjects. The physically active subjects stayed physically active and served as controls (80). Subject numbers in each group were as follows: old, active n= 14; old, inactive n=17; young, active n=15; young, inactive n=14. * indicates post-training significantly lower than pre-training ($P<0.05$).

These findings were supported in a later paper by the same group and extended to show that impaired MAPK signaling reduced NF-κB activation in older mice (10). Renshaw et al. (65), on the other hand, observed substantially lower mRNA expression for TLR1-9 in both splenic macrophages and thioglycollate-stimulated peritoneal macrophages from older (18-24 months), compared to younger (2-3 months), C57BL/6 mice. These authors also reported significantly lower macrophage-surface expression of TLR4 in older mice (65).

There were no differences in TLR4 expression between younger (18-35 years of age) and older (65-80 years of age) humans (80) (Figure 5) who were screened to exclude several chronic diseases and drugs known to influence inflammatory processes. We are unaware of other published papers which have examined possible differences in TLR expression between young and old people.

Exercise, ageing and TLR4

Few studies have been conducted to examine the influence of physical activity or exercise training on TLR in older subjects, and these studies were all conducted by the same research group (20, 46 ,47, 80). Early research, undertaken to explain a training-induced lowering of LPS-stimulated inflammatory cytokine expression, showed significantly lower TLR4-mRNA in whole blood samples from older resistance trained, compared to age-matched untrained, subjects (20). In follow-up studies, physically active older adults had significantly lower CD14+ cell-surface TLR4 expression than physically inactive subjects of the same age and health status (4647).

As aforementioned, physical activity level appeared to be more important than age group (young: 18-35 years; old: 65-80 years) with respect to TLR4, CRP, and LPS-stimulated inflammatory cytokine production (46). Nevertheless, we found that 10-12 weeks of exercise training reduced TLR4 expression of both young and old sedentary people to the level found in physically active controls (80) (Figure 6). These changes occurred concomitantly with lower CRP (unpublished data), and marginally lower inflammatory cytokine production (80), but we are as yet unable to determine whether a training-induced blunting of TLR4 represents a positive change in terms of long-term health status.

TLR polymorphisms and age/disease

We assume that an exercise training-induced lowering of TLR4 signaling is a positive adaptation to training in older adults as it represents an adaptation that could lead to reduced systemic inflammation and improved overall health. Single nucleotide polymorphisms of TLR4, such as the Asp299Gly allele, provide an interesting model for comparison, since the Asp299Gly allele and high levels of physical activity are both associated with hyporesponsiveness to LPS (46, 47, 71). The Asp299Gly polymorphism is associated with a reduced incidence of cardiovascular disease (14, 38) and it has been shown that a higher proportion of centenarians possess this allele 5). Kolek et al. (38) suggested, after finding significantly lower CRP and lower incidence of myocardial infarction in subjects with the Asp299Gly allele, that "…down-regulation of innate immune responsiveness could beneficially modify CAD[CLA9] and diabetes risk and might provide a novel basis for genetic risk stratification and therapeutic targeting." Put into an antagonistic pleiotropy context, those with the wild type A allele (about 90 % of the Caucasian population) (5) are protected from early life bacterial infection but are at greater risk of death from diseases linked to inflammation, presumably from an overly aggressive inflammatory response, in later life (69).

TLR4 polymorphisms are also known to affect diabetic outcomes. For example, patients with type 2 diabetes possessing the Asp299Gly allele were less likely to have severe neuropathy than patients with the wild type allele (67). In contrast, these researchers found no differences in diabetic nephropathy between patients with the wild type and the Asp299Gly allele (67) and one group found no influence of TLR4 allele on diabetic outcomes (31).

Both TLR4 polymorphisms and exercise training are associated with hyporesponsiveness to LPS (20, 47, 71) and improvements in chronic disease outcomes (14, 23, 38, 76). Therefore, we speculate that exercise training-induced lowering of TLR4 is a positive adaptation in an older population.

Summary and conclusions

The purpose of this review was to summarize the research which has examined the effects of acute and chronic exercise on cell-surface TLR expression. The TLR pathway plays an important role in mediating whole body inflammation, which has been implicated in the development of chronic disease as well as acute disturbances of

immunity. TLRs have been reported to play roles in host defence against microbial pathogens (2, 40) and inhibition of the suppressor actions of CD4+25+ T-cells (58). The key product of TLR signaling is the production of inflammatory cytokines and proteins, which have been implicated in the pathophysiology of cardiovascular disease, type II diabetes mellitus, asthma, coronary heart disease, inflammatory bowel disease, and rheumatoid arthritis (15). An accumulation of chronic, low-grade inflammation is common in individuals that live a sedentary lifestyle; however, the mechanism underlying this connection is not fully understood. Based on the existing scientific literature, it appears that TLRs may be involved in the link between a sedentary lifestyle, inflammation, and disease.

Both acute aerobic and chronic resistance exercise have been reported to decrease monocyte cell-surface expression of TLRs (42, 80). Although another study found no effect of an acute bout of resistance exercise on monocyte cell-surface TLR4 expression (47), the most likely explanation for the difference in these findings is related to the severity/duration of the exercise stimulus and the age of the subject population. Others have reported that a period of chronic exercise training decreases inflammatory cytokine production and the study by Stewart et al. (80) was the first to report that cell-surface TLR4 expression decreased as well.

The exact physiological stimulus mediating an exercise-induced decrease in cell-surface TLR expression is not known; however, a number of possible signals have been implicated. In this review, we summarized the effects that have been attributed to anti-inflammatory cytokines (i.e. IL-4, etc.) (50, 74, 78) and stress hormones (i.e. glucocorticoids, etc.) (22, 32, 41, 42). The physiological stress associated with exercise directly affects the stress hormone release that occurs. These effects may contribute to post-exercise immunodepression and the reported higher susceptibility to infection in athletes. In the long-term, a decrease in TLR expression may represent a beneficial effect because it decreases the inflammatory capacity of leukocytes, thus altering whole body chronic inflammation.

Chronic inflammation has been implicated in the development of a number of different disease states (13, 30, 77) and leukocytes with high cell-surface TLR expression mostly account for this elevated inflammation. The exact stimulus by which exercise decreases cell-surface TLR expression is not known. More research is needed to identify and examine this response. Also more studies are needed to confirm previous studies from our laboratories. Future research in the area of exercise and TLR expression should develop mechanistic methodology to evaluate this pathway.

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