Toll like receptor expression induced by exercise in obesity and metabolic syndrome: A systematic review

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ABSTRACT

Background: *Obesity and metabolic syndrome are disorders that correlate with the activation of pro-inflammatory pathways and cytokine production, to which Toll like receptors (TLR) contribute. Exercise may act as an anti-inflammatory modulator, but there is no consensus about the role of the TLR in this tuning.*
The present styudy aims to systematically review the current evidence on exercise-induced TLR regulation in animals and humans suffering from obesity and metabolic syndrome.
Methods: *Pubmed and Scopus databases were searched for publications from 1990 to September 2015. Search terms included: "Toll like Receptor", "TLR", "exercise", "obesity", "diabetes", and "metabolic syndrome". Elegibility criteria comprised: randomized control trials, cross-sectional and cohort studies; human or animal models with metabolic syndrome; any type of exercise; TLR expression measurement in any tissue by a clearly reported technique. The quality of selected studies was assessed using a modified version of the Downs and Black Quality Assessment Checklist. Data of study design; population; exercise type, timing and training elements; measurement technique, tissue analyzed and main outcome were extracted and categorized to facilitate data synthesis.*
Results: *17 studies were included, of which 11 publications obtained a high, 5 a moderate and 1 a low score for quality assessment. A total of 8 human studies were analyzed: 6 studies used endurance continuous or interval training protocols, 1 study resistance training and the remaining study was performed following a marathon race. Blood cells were analyzed in seven studies, of which four studies sampled peripheral blood mononuclear cells (PBMC), three analyzed whole blood and one study sampled skeletal muscle. Nine animal studies were included: 8 used endurance training and 1 acute aerobic exercise. A variety of tissues samples were explored such as PBMC, skeletal muscle, adipose, vascular and nervous tissue. Globally, the animal studies showed a marked tendency towards a down-regulation of TLR2 and 4 expression accompagnied with, a reduced activation of nuclear factor-kappaB (NF-κB) signaling and cytokine production, and an improvement in insulin sensitivity and body composition.*
Conclusion: *While animal studies showed a marked tendency towards TLR2 and 4 down-regulation after chronic endurance exercise, the current evidence in human is not sufficiently robust to conclude any role of TLR in the anti-inflammatory properties of exercise.*

Keywords: TLR2, TLR4, nuclear factor-kappaB, chronic inflammation, immunomodulation, cytokines, diabetes, metabolic disorders

INTRODUCTION

Overweight and obesity have become increasingly prevalent. According to the World Health Organization (WHO) approximately two billion adults are overweight and over half a billion are obese (37). Over half the adult population in developed countries is either overweight or obese (14). Obesity prevalence is directly associated to the country income level (37). As it is a main risk factor for metabolic co-morbidities related to premature death (32), obesity is a key public health concern. Associated endocrine and metabolic alterations lead to the development of the metabolic syndrome (MetS), a collection of medical conditions including insulin resistance, type 2 diabetes mellitus, dyslipidemia, hypertension, obstructive sleep apnea and non-alcoholic fatty liver disease (14).

Obesity is often associated with a chronic low-grade inflammation state caused by a pathological expansion of the adipose tissue, leading to elevated levels of free fatty acid (FFA). Activation of innate immune receptors, such as Toll like receptors (TLR), in obesity and MetS is possibly part of the chronic pro-inflammatory process (11). TLR are pattern-recognition receptors (PRR) expressed in different cell compartments. In mammals, there are 12 known members involved in detection and immune response to diverse exogenous signals such as pathogen-associated molecular patterns (PAMP), as well as endogenous ligands like damage-associated molecular pattern molecules (DAMP) involved in sterile inflammation induced by tissue damage and cellular stress (15). Within the recognized ligands, FFAs and endotoxin like lipopolysaccharides (LPS), activate I kappa B kinase (IKK), nuclear factor-kappa B (NF-κB) signaling (1) and also the mitogen-activated protein kinase (MAPK) pathway (12).

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TLR activation may have an important protective role against viral infections when detecting gram- negative and gram-positive bacteria (1). However, in obesity conditions the majority of animal studies have reported increased TLR expression associated to a pro-inflammatory state (11). Inversely, suppression of TLR2 and TLR4 expression or their deficit reduces chronic inflammation, suggesting a pathogenic role for TLR in obese animal model (11). In humans with obesity and type 2 diabetes, elevated TLR activity has been correlated with the severity of insulin resistance (15). Exercise modulates TLR expression in monocytes, adipose tissue and skeletal muscle, which could be a molecular mechanism by which exercise exerts its anti-inflammatory effect (8). Reduced TLR4 expression at the cell surface of leukocytes has been found in young and old physically active individuals, in whom cytokine production following LPS stimulation was lower compared with sedentary participants (21). Moreover, exercise reduces plasma FFA levels, thereby decreasing TLR activity (32).

Exercise is a well-known tool to counteract the chronic pro-inflammatory state and the consequences of metabolic syndrome but the mechanisms are not totally elucidated, certainly when focusing on TLR signaling. Exercise is able to modify TLR expression in some conditions in both human and animal models (9). However, currently no consensus exists regarding exercise-induced TLR regulation. This mechanism possibly reduces the low-grade inflammation that often accompanied obesity and MetS. Hence, the present study aims to systematically review the current evidence on TLR expression in any tissue of either obese/MetS animals or humans undergoing an exercise program. We aim to better understand how exercise affects immune signaling and to set a precedent that could contribute to the development of guidelines related to physical activity that could be useful for clinical practitioners.

METHODS

This systematic review was conducted using the PRISMA statement for reporting systematic reviews (19).

Search strategy and study selection

In September 2015, the electronic databases PubMed and Scopus were queried. The following search terms were used as keywords: “Toll Like Receptor”, “TLR”, “exercise”, “obesity”, “obese”, “diabetes”, and “ metabolic syndrome” using the Boolean connectors AND/OR respectively. The query was limited to articles published between 1990 - September 2015 in English, Spanish, Portuguese and French.

The following criteria were applied: 1) Population type: human or animal with metabolic syndrome, e.g., obesity, diabetes, hyperglycemia, hypertension, dyslipidemia, and atherosclerosis (11). No age restriction was applied and all activity levels (active or sedentary) were admitted. 2) Study type: randomized control trials, cross-sectional studies and cohort studies. 3) Exercise type: any type of exercise, e.g., endurance and/or resistance training. 4) Outcomes type: all types of Toll like receptor expression, e.g., mRNA or protein in any tissue, measured by a clearly reported technique.

After removing duplicates, IR began the study selection process by screening titles and abstracts. Subsequently, the full text versions of the selected publications were reviewed in detail by IR and HZ to ensure the existence of metabolic syndrome in the studied subjects. Populations with pathologies unassociated to metabolic conditions were excluded. Alternative interventions in addition to exercise were admitted, such as pharmacological administration, TLR agonist, insulin clamps, and pathogen injection. The papers were discussed by IR and HZ and discrepancies were resolved in consensus.

Data collection and synthesis

IR extracted the data from the selected studies and HZ checked these data. The template used was classified by population type (human or animal) and sorted by the study characteristics (author, year and study type). The following information was provided: participants’ characteristics (sample number, sex-age distribution and metabolic disorder); exercise intervention type (e.g., endurance, or resistance); timing (e.g., acute or chronic); training elements (duration, frequency, intensity, volume, and mode); measurement technique; tissue analyzed; and main outcome. A qualitative synthesis was performed with the extracted data; the quantitative analysis was not feasible given the diverse analytical techniques to report the main outcome.

Quality assessment

The Downs et al. checklist (6) adapted by Munn et al. (23) was selected to measure the quality of the included (non)-randomized studies. The Munn’s list (2010) was complemented with items from the original list and some descriptions were adapted to be applicable to both human and animal studies based on the Gold Standard Publication Checklist to improve the Quality of Animal studies (10). In animals experiments, the characterization was evaluated by specific criteria (species, genetic background, origin and source) added in the third item of the checklist. The item 4 of the original Downs et al. checklist (1998) was re-included to obtain the duration, frequency, intensity, volume and mode of exercise; for animal studies, the time schedule, housing, nutrition and drug administration were also obtained (10). The confounding variables that had to be reported to score the item 5 in human studies were sex and age. In case of animal models, the weight at the beginning of the experiment had to be stated as well. To consider the number and characteristics of patients lost during follow-up, the original Item 9 of Downs et al. (1998) was reincorporated. The Item 11 was modified to include the assessesment of source, recruitment, and selection procedure. The items 12, 21 and 22 of Munn et al. (2010), for which a representative sample from the entire population is required, were discarded because the participants have to be screened to ensure the presence of metabolic disorder. For animal models, the sample size calculation had to be reported and had to include the minimum of animals required by the calculation (10).

The original Item 13 of the Downs et al. (1998) checklist was adapted and its description complemented to verify intervention supervision and the proper performance of the exercise. Item 15 of the Downs et al. (1998) checklist was incorporated to ensure that the data examiner was blinded from the charac-

teristics of the subjects tested (name, sex, age, and activity level). Item 16 of the Munn et al. (2010) list was omitted since the data dredging was relevant for the present study. Item 20 demands a standardized technique to be clearly described to ensure the validity and reliability of the main outcome. The original Item 24 of the Downs et al. (1998) checklist was re-included to ensure the randomization of the intervention groups. Item 25 demands separate analysis to ensure adequate adjustment for confounding analyses in the presence of significant differences in sex, age, or weight at the beginning of the trial. Supplementary 1 contains the checklist template adapted for this systematic review and the description of the items.

The Downs et al. (1998) scale was used to score each item. Questions were answered "YES" if the description requested was provided (score = 1); and "NO" (score = 0) when no report was given or was unclear. IR checked every item, filling a template with data extracted from the paper to support the assigned rate. The results were discussed with HZ to assign the final scores. Study quality was determined using Munn's scoring scale (23): positive answers were summed and converted to a percentage: high quality studies scored over 70%, moderate between 60-74%, and low quality scored under 60%.

Results

Literature Search

Querying both databases generated 477 records, of which 214 duplicates were removed. The remaining 263 titles and abstracts were screened. One hundred ninety-four did not meet the eligibility criteria: 1 by language, 1 by year of publication, 42 by study design, 134 by intervention (no exercise training), 16 by outcomes (no TLR expression report in analyzed tissue). Sixty-nine full texts were reviewed to confirm eligibility and the presence of metabolic syndrome in the studied populations, upon which 52 studies were excluded: 41 for being healthy, 2 due to chronic fatigue, 2 due to brain ischemia, 1 due to Alzheimer's disease, 1 due to lower back pain, 1 due to induced hypoxia, 1 due to severe liver injury, 1 due to myopathy, 1 due to myositis, 1 due to stroke. Seventeen records met the eligibility criteria and were included for qualitative synthesis (Figure 1).

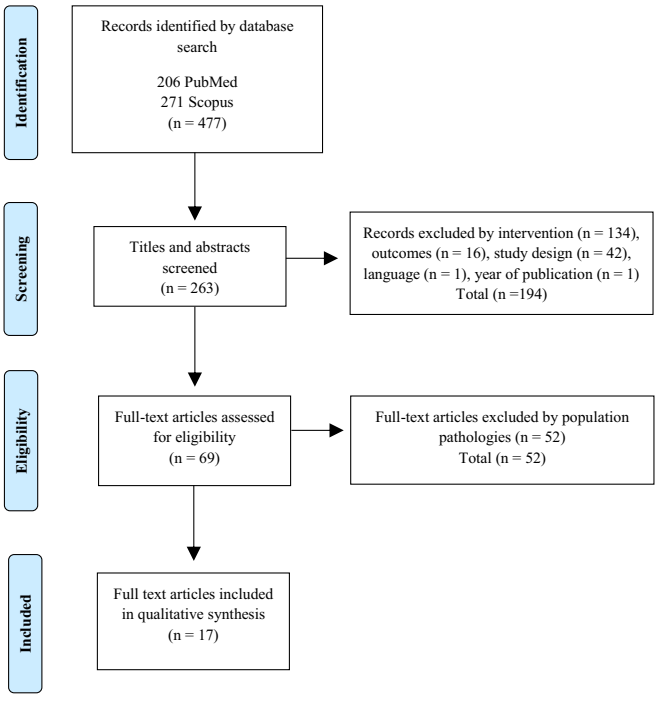

Figure 1. Search results through the review process

Quality assessment of studies included

Among the 17 studies, the quality scores ranged from 56 - 94% (Supplementary 2). Eleven publications obtained a high score, five a moderate and one a low score. All publications had clear descriptions of the hypothesis/objective. The main outcome to be measured, related to Toll like receptors and exercise was mentioned within the introduction or methods in 16 papers. The participant source and inclusion/exclusion criteria were reported in 5 of the 8 human studies. In animal studies, the origin, source, species and genetic background was mentioned in 8 of 9 studies. Exercise intervention type and its protocol was described in 7 of 8 human publications; while in animal studies this information was detailed with time schedule, housing and nutrition in 7 of 9 studies. The distribution of principal confounding variables such as sex and age were stated in 7 of 8 human studies; besides the distribution of these variables in animals, the initial weight was provided only in 3 of 9 studies.

All 17 studies described simple outcome data from findings associated with Toll like receptors and exercise interventions. The 17 papers reported estimates of random variability (standard error/standard deviation), probability values, and number/characteristics of patients lost during follow up. Of the 8 human studies, 4 papers did not report the participants´ source or selection procedure while the remaining four clearly described the selection process by screening. Four of the nine studies in animal calculated the sample size and included the minimum sample amount. The exercise interventions were supervised by an examiner or monitored in all 17 studies. Internal validity by blinding the examiner from participants' characteristics was reported in 3 of the 17 publications. Appropriate statistical analyses were performed in 9 of 17 studies. Toll like receptors expression measurement technique and the tissue analyzed was stated in all 17 studies. Randomization of the intervention groups was specified in 13 of 17 papers, and an adequate adjustment for confounding variables within the groups was performed in 11 of 17 publications.

Characteristics of included studies

Methods

The main characteristics of the 17 selected studies are presented in Table 1 and Table 2. All 17 studies were intervention studies, of which 12 were randomized control trials (RCTs), 4 non-randomized control trials (NRS) and one quasi randomized trial (qRCT). All publications were in English and published between 2010 and 2015. Eleven studies were from the Americas: 7 from the United States, 3 from Brazil, and one from Canada. The remaining 7 studies were European or Asian: 3 from Germany, 3 from China and 1 from Japan.

Table 1: Summary of the 8 human studies included in the systematic review

Human studies							
Reference	**Study type**	**Population types and conditions**	**# Subjects, gender and age**	**Exercise type**	**Timing/duration of intervention and intensity**	**Analytical technique/ blood cell type or tissue**	**Primary outcome**
Liu et al, 2015(20)	RCT	• Type 2 diabetic (AR/DCT) • Healthy control subjects (CTL)	Total: n=62 • AR+DCT: n=42, 30F + 12M, 52.6y • CTL: n=20; 13F + 7M, 51.2y	Chronic endurance and resistance training with elastic band	• AR: aerobic ex 12 weeks, 3d/wk, 10-30 min at 40-60% VO_2max + resistance ex 2-3d/wk, 2 sets of 8-10 repetitions at 50-60% 1RM • DCT: drug therapy and diet control • CTL: No intervention	• Real-time quantitative PCR • Western blot • PBMC (Mononuclear blood cells)	• Before treatment: higher TLR4 mRNA and protein expression levels in AR and DCT than in CTL. • After exercise intervention: TLR4 mRNA and protein expressions were significantly lower in AR group than DCT group.
Coen et al, 2010(5)	RCT	• Hyperchol esterolemi c physically inactive subjects (R/RE) • Hyperchol esterolemi c physically active subjects (ACTL)	Total: n=49 • R: n=17, 9M + 8F, 52.1y • RE: n=16, 7M + 9F, 52.2y • ACTL: n=16, 7M + 9F, 51.6y	Chronic endurance (treadmill and walking) and resistance training (leg press, leg extension, leg curl, chest press, lat pull-down, seated row, leg adduction, and leg abduction).	• R: 20 weeks of rosuvastatin calcium (10mg/d) treatment • RE: same as R with the last 10 weeks including ex training (3d/wk). A session consisted in 20min at 60-70% of HR reserve + 2 sets of 8 resistance exercises at 80% 1RM • ACTL: No intervention	• Color flow cytometry • Whole blood	• Higher TLR4 expression after treatment in R. • Percentage of $CD14^+$ monocytes expressing TLR4 was not different between and within R, RE and ACTL throughout the study. • More TLR4 expressed in pro-inflammatory than classic monocytes.
Lambert et al, 2008(16)	RCT	• Sedentary obese elderly subjects • AR/DCT	Total: n=16 • DCT: n=8, 4F + 4M, 69.6y • AR: n=8, 4F + 4M, 68.5y	Chronic endurance (treadmill, step-ups, climbing, stationary cycling, stair-master exercise) and resistance training (squats, leg press, knee extension, knee flexion, row, seated chest press, biceps curl, and triceps extensions)	• AR: 12 weeks (3d/wk), endurance ex for 20min at 75-90% HRpeak + 30-40min resistance ex at 65-80% 1RM (6-12 repetitions) • DCT: 12 weeks of weight loss therapy (energy deficit diet and behavior therapy)	• Real-time quantitative PCR • Muscle vastus lateralis	• AR reduced TLR4 mRNA. • Expression remained unchanged in DCT.
Nickel et al, 2011(25)	NRS	• Obese amateur marathon runners (ONE) • Lean non elite (LNE) • Lean elite (LE)	Total: n=47, M, 40y • ONE: n=15 • LNE: n=16 • LE: n=16	Chronic endurance training	• 10 weeks (4d/wk) of continuous endurance and interval training • Intensity given by individual target HR • ONE and LNE: 40km/wk; LE: 55km/wk	• Real-time quantitative PCR (all groups) and western blot (only in LNE) • PBMC	• Before training: TLR2, 4 and 7 mRNA: no differences between the groups. • After training: higher TLR2 mRNA in LNE, up-regulation of TLR4 and 7 mRNA in all groups. • No exercise-induced changes in MyD88mRNA gene-expression between the groups. • In LNE, increase in TLR4 and 7 protein expression after training, no change in TLR2 and MyD88. No protein analysis in ONE and LE.
Robinson et al, 2015(33)	RCT	• Sedentary pre-diabetic subjects • HIIT/MIC T	Total: n=38 • HIIT: n=20, 17F + 3M, 52y • MICT: n=18, 14F + 4M, 52y	Chronic endurance training (cycle ergometer, treadmill, outdoor walking, elliptical training)	• HIIT: 10 sessions spread over 2 weeks consisting in 4-10 repetitions of 1-min intervals at 85-90% Wpeak and 1-min rest at 20% Wpeak • MICT: 10 sessions spread over 2 weeks consisting in 20-50 min of continuous training at 32.5% Wpeak	• Flow cytometry • Whole blood	• Decreased TLR4 expression in lymphocytes and $CD14^+$ monocytes in both MICT and HIIT. • Decreased TLR4 expression in $CD15^+$ neutrophils only in MICT, no change in HIIT. • TLR2 expression was reduced in both MICT and HIIT in lymphocytes but not in monocytes and neutrophils.

Reyna et al, 2013(31)	NRS	• Sedentary and lean subjects (LE) • Obese (OB) • Type 2 diabetic (T2DM)	Total: n=36 (no gender distribution reported) • LE: n=17, 39y • OB: n = 8, 40y • T2DM: n=11, 50y	Chronic endurance training (cycle ergometer)	• 40min/d of endurance ex for 15 consecutive days • Each session consisted in 4 x 8min at 70% VO_2peak followed by 2min of rest • First session within the week after insulin clamp, which was repeated 36h after last session	• Western blot • PBMC	• Before intervention higher TLR4 protein content in T2DM and OB. • After ex training and insulin infusion: no change in TLR2 and 4 expressions in any group.
Nickel et al, 2012(24)	NRS	• Obese amateur marathon runners (ONE) • Lean non elite (LNE) • Lean elite (LE)	Total: n=47, M, 40y • ONE: n=15 • LNE: n=16 • LE: n=16	Acute endurance bout (marathon)	• Marathon race	• Real-time quantitative PCR (all groups) and western blot (only in LNE) • PBMC	• Immediately after race: no change in TLR2 mRNA levels in any group, down-regulation in TLR4 in only LNE group, TLR7 mRNA levels decreased in all groups. No change in TLR2, 4 and 7 protein in LNE, no protein analysis in LE and ONE. • 24 hours post-race: no change in TLR2 mRNA levels in any group, increase in TLR4 and 7 mRNA levels in all groups. Only TLR7 protein expression decreased in LNE, no protein analysis in LE and ONE.
Philips et al, 2012(28)	qRCT	• Obese post-menopausal women intervention (RE) • Obese post-menopausal women control (OCTL)	Total: n=23, F • RE: n=11, 64.8y • OCTL: n=12, 66.4y	Chronic resistance training (chest press, shoulder press, and seated row or cable press, abdominal crunches, and back extensions)	• RE: 12 weeks (3d/wk), 3 sets of 10 repetitions at 100% of 8RM • OCTL: stretching, health education and safety talks • Test: 1 week after last session: a single resistance bout with 3 sets of 10 exercises at 8 RM with 1.5min recovery	• Real-time quantitative PCR • Whole blood • Subcutaneus adipose tissue (SCAT)	• TLR4 mRNA expression in whole blood was not different between the groups after interventions. • After RE intervention: TLR4 mRNA expression remained unchanged in SCAT. • Total volume load was negatively correlated to TLR4 mRNA in SCAT. • RE improved body strength and reduced circulating CRP, leptin and TNF levels

RCT: Randomized Control Trial; NRS: Non-Randomized Control Study; qRCT: quasi-Randomized Control Trials; ex: exercise; min: minutes ; wk: week; d: day; h: hour; HR: heart rate; Wpeak: peak power output; VO2max: maximum oxygen consumption; VO_2peak: peak oxygen uptake; 1RM: One Repetition Maximun; m/min: meters per minute; PBMC: peripheral blood mononuclear cells; TLR: Toll Like Receptor; mRNA: Messenger RNA; CRP: C-Reactive Protein; TNF: Tumor Necrosis Factor; MyD88: Myeloid differentiation primary response gene 88.

Table 2: Summary of the 9 Animal studies included in the systematic review

Animal studies							
Reference	**Study type**	**Population types and conditions**	**# Animals, sex and age**	**Exercise intervention type**	**Timing/duration of intervention and intensity**	**Analytical technique/ blood cell type or tissue**	**Primary outcomes**
Li et al, 2015(18)	RCT	• Sprague Dawley rats • Diet induced obesity • ND/ND-EX/DIO-S/DIO-EX	Total: n=122, male no age reported • RD-S: n=31 • RD-EX: n=29 • DIO-S: n=30 • DIO-EX: n=32	Chronic endurance (treadmill running)	• ND: regular diet • S: sedentary • DIO: high fat diet for 12 weeks • EX: 8 weeks, 5d/wk, 60min/session, 18m/min	• Western blot • Muscle soleus • Muscle ventricle	• Higher protein and mRNA levels of TLR2 and 4 in soleus and ventricule of DIO-S versus ND-S and DIO-EX. • TLR2 and 4 protein and mRNA levels were lower in DIO-EX compared with ND-EX and DIO-S.

Wu et al, 2014(38)	RCT	• ApoE null C57BL/6J mice • Diet induced obesity • AS/AD/AEX	Total: n=36 • 10 week old male mice • no specific amount of mice per group	Chronic endurance training (treadmill)	• AS: atherosclerotic control mice • AD: 12 weeks of 10mg/kg simvastatin treatment • AEX: 12 weeks, 5d/wk, 60min/session, 13m/min (zero slope)	• Immunohistochemistry • Vascular tissue	• TLR4 protein expression was increased in AS. • TLR4 protein expression reduced in AD and AEX groups, with a larger reduction in AD compared with AEX. • Increased expression of miR-146a in both AD and AEX.
Carpenter et al, 2012(3)	RCT	• CD1 mice • Diet induced obesity • CTL-DIO/FEX/VEX and CTL non DIO	Total: n=24 • 6 male mice/group • 2-14 weeks at start of DIO (CTL-DIO/FEX/VEX but not CTL non DIO), 64-66 weeks at start of low-fat diet (LFD, 4 groups) + EX (FEX/VEX) for 8 weeks	Chronic endurance training (forced-treadmill running and voluntary wheel running)	• CTL-non DIO: normal diet, no exercise • CTL-DIO: induced obesity, no exercise • FEX: 8 weeks, 5d/wk (Monday to Friday), forced treadmill running, 60min at 12 to 20 m/min • VEX: 8 weeks, 5d/wk, (Monday to Friday) voluntary wheel running with 24h access	• Flow cytometry • PBMC (Monocytes blood cells)	• Before LFD, the expression of TLR4 in CTL non DIO was higher than in DIO. • After LFD, TLR2 expression was lower in VEX than in the other 3 groups. TLR4 expression was lower in VEX than in CTL non DIO and FEX.
Kawanishi et al, 2010(13)	RCT	• C57BL/6 mice • Diet induced obesity • non DIO/non DIO-EX/DIO/DIO-EX	Total: n=38, 4 wk-old • ND: n=7 • ND-EX: n=7 • DIO: n=12 • DIO-EX: n=12	Chronic endurance training (treadmill running)	• ND: regular diet with standard chow • DIO: high fat diet for 16 weeks • EX: 16 weeks, 5d/wk, 60min/session, 12-20m/min	• Real-time quantitative PCR • Adipose tissue	• TLR4 mRNA levels varied as an effect of diet and exercise training alone as well as diet combined with exercise. • Differences in TLR4 expression between ND vs DIO and DIO vs DIO-EX. • Down-regulation of TLR4 mRNA levels after DIO-EX compared with DIO.
Esposito et al, 2010(7)	RCT	• Outbred CD-1 mice • Diet induced obesity • DIO/DIO-EXE/ND	Total: n=36 • 12 male mice/group • 6-8 week-old	Chronic endurance training (treadmill running)	• DIO: high fat diet for 6 weeks • DIO-EXE: 6 weeks of DIO + ex as follows: 5d/week, 1h/session, 21-22 m/min, 1% grade • ND: normal diet with standard chow, sedentary	• Flow cytometry • intensity • PBMC (Monocytes blood cells)	• At the end of the intervention, DIO expressed more TLR4 than DIO-EXE and ND, with no difference between the latter two.
Zhou et al, 2011(39)	NRS	• C57BL/6J mice • Diet induced obesity • ND/DIO/OB-ND-EX	Total: n=20 • 4 mice/group • 6 week-old • no sex distribution	Chronic endurance training (treadmill)	• ND: standard chow • DIO: long-term high fat diet induced obesity • DIO-ND-EX: long-term high fat diet and switch to normal diet plus exercise for 4 weeks (5d/wk, 1h/session, 12m/min)	• Real-time quantitative PCR • Bone marrow-derived macrophages	• Lower TLR2 but higher TLR4 mRNA levels in DIO and DIO-ND-EX compared with ND, with no difference between DIO and DIO-ND-EX. • Exercise did not reverse the decrease in TLR2 mRNA levels in response to infection in DIO mice.

Oliveira et al, 2011(27)	RCT	• Wistar rats • Diet induced obesity • ND/DIO/DIO-EX/DIO-AEX	Total: n=40 • 8 week-old male rats • 10 rats/group	Chronic and acute endurance training (swimming)	• ND: normal diet with standard chow • DIO: high fat diet for 20 weeks • EX: 8 weeks, 5d/wk, 1h/session, progressive load increase up to 5% of body weight • AEX: 2 moderate bouts of 3 hours separated by one rest period of 45 min	• Real-time quantitative PCR and western blot • Skeletal muscle, liver and adipose tissue	• TLR4 protein expression was higher in DIO than in ND in all tissues. • After chronic training: DIO-EX group had decreased mRNA and protein TLR4 levels compared with DIO in all tissues. • After acute exercise: no changes in TLR4 protein expression in all tissues. Decrease in TLR4 mRNA levels only in skeletal muscle. • Reduction in TLR4/MyD88 interaction in both EX and AEX compared with DIO sedentary rats.
Ropelle et al, 2010(34)	RCT	• Wistar rats • Diet induced obesity • ND/DIO/DIO-EX	Total n=32-40 • 8-10 male rats/group • ND/DIO/DIO-EX: 4 week-old	Chronic and acute endurance training (swimming and treadmill)	• ND: normal diet with standard chow • DIO: high fat diet for 12 weeks • DIO-EX-A: acute single bout of 60min at 10-15m/min with 5% inclination. • DIO-EX-C: 4 weeks, 5d/wk, 1h/session, 2% body weight overload	• Western blot • Hypothalamus tissue	• DIO induced an increase in TLR4 expression. • Acute exercise did not reduce TLR4 expression in DIO. • No TLR expression report after chronic training, but reduced IKKb phosphorylation and increased IκBα expression in the hypothalamus of obese rats.
Oliveira et al, 2013(26)	RCT	• Wistar rats • Diet induced obesity • ND/DIO/DIO-EX	Total: n=24 • 8 male rats/group • 6 week-old	Acute endurance bout (swimming)	• ND: normal diet with standard chow D • DIO: high fat diet for 12 weeks • DIO-EX: 2 moderate bouts of 3h separated by one rest period of 45 min	• Western blot • White adipose tissue	• Higher interaction in TLR4/MyD88 in DIO compared with ND. • DIO-EXE reduced this interaction compared with DIO.

RCT: Randomized Control Trial; NRS: Non-Randomized Control Study; DIO: diet-induced obesity; ex: exercise;min: minutes; m: meter; wk: week; d: day; h: hour; HR: heart rate; m/min: meters per minute; ApoE: Apolipoprotein E; TLR: Toll Like Receptor; mRNA: Messenger RNA; TNF: Tumor Necrosis Factor; MyD88: Myeloid differentiation primary response gene 88; IKKb: I kappa B kinase b; IκBα: inhibitor of nuclear factor-kappa B

Participants

The included publications comprised 271 human subjects: 131 female and 104 male subjects. One study of 36 participants did not specify gender. Age ranged between 39 and 69.6 years. Five of the eight studies included obese subjects, two of which were based on an elderly population, two on obese amateur runners, and one on type 2 diabetic participants. One study was based on subjects solely diagnosed with type 2 diabetes. Another one set pre-diabetes as inclusion criteria, and the last one studied subjects with hypercholesterolemia. Two studies used the same sample of subjects and thus only one sample set was included (24). The inclusion of 348 animals was clearly reported, however one study did not specify the accurate total sample number, which approximately comprised 32 to 40 animals (4 groups comprising 8-10 animals per group). The animals' age ranged from 4 to 66 weeks at the start of the training intervention. All animal studies used high fat diet induced obesity as a metabolic condition. Moreover, one study induced atherosclerosis.

Human studies

Of the 8 selected human studies, 7 used an endurance exercise training protocol. Chronic long-term endurance protocols were applied in 4 studies, lasting 10-12 weeks, at a frequency of 3-5 days per week. Training sessions lasted initially 10 minutes and continued for another 20-50 minutes. The intensity was set between 60-70% of heart rate reserve (5), 40-60% of maximum oxygen consumption (VO_2max) (20) or 75-90% of peak heart rate (16). One study did not state the training

session duration, only that intensity was gradually increased up to an individual target heart rate (25).

Moreover there were two short-term intervention studies with 10 and 15 sessions of endurance exercise distributed over two weeks. One study was designed with two groups performing either 20 minutes of continuous exercise or intervals (33). The other short endurance protocol study included four cycles of 8:4 work ratio (31). These endurance training sessions were performed on a treadmill, elliptical trainer, step-ups, cycle ergometer, or by climbing stairs or walking outdoor. Furthermore, other exercise types such as marathon race were used to evaluate obese amateur runners (24). Resistance training combined with endurance exercise sessions was used in three studies (5, 16, 20). Muscle strengthening was performed using free weights or elastic bands, and the intensity was set between 50-80% of one repetition maximum (1RM). Finally, one remaining study exclusively used resistance training for 12 weeks with a frequency of 3 days per week at an intensity corresponding to 8RM. The participants completed two sets of 8 repetitions and the last set to fatigue on 10 different types of weight machines (28).

Animal intervention studies

Eight of the nine animal studies used chronic endurance training. Intervention duration ranged from 4 to 16 weeks, with a frequency of 5 days per week. The majority of the exercise sessions lasted 1 hour per day (3, 7, 13, 20, 27, 34, 38, 39). One study included a group performing voluntary wheel running (3). When exercise was imposed, the workload was set at 12 to 20 m/min on a treadmill (3, 7, 13, 18, 38, 39). Supervised chronic swimming exercise was performed with a load equal to 2% (34) and 5% of body weight and progressively increased (27). In those studies, measurements were also made after an acute bout of exercise: after 1 hour at 10–15 m/min with 5% of body weight (34) or following 2 swimming bouts of 3 hours separated by 45 minutes recovery (27). The latter protocol was also used in a study using acute aerobic exercise only (26).

Analytical technique/blood cell type or tissue

Different standardized techniques were used to analyze TLR expression. Of the human studies, five used real time quantitative polymerase chain reaction (qPCR) (16, 20, 24, 25, 28), two used color flow cytometry (5, 33) and three western blotting (24, 25, 31). Blood cells were analyzed in seven studies, of which four studies sampled peripheral blood mononuclear cells (PBMC) (20, 24, 25, 31), three studies sampled whole blood (5, 28, 33). Moreover, other tissues such as subcutaneous adipose tissue (28) and muscle vastus lateralis (16) were sampled in the remaining two human studies.

In the animal studies, four studies used western blot (18, 27, 34), three qPCR (13, 27, 39) and three color flow cytometry (3, 7), or immunohistochemistry (38). Blood was sampled in two studies analyzing PBMC (3, 7), whereas three assessed the adipose tissue (13) (26, 27) and two the skeletal muscles (18, 27). The remaining studies sampled the hypothalamus (34), liver, bone marrow-derived macrophages (39), and vascular tissue (38) and ventricle muscles.

Secondary outcomes

Besides determining TLR expression in control conditions and after exercise, additional measurements were taken in the 17 studies. The following assessments were made with regard to metabolic syndrome risk factors: body mass index (BMI) (5, 16, 24, 25, 28, 31, 33), waist circumference (24, 25), fat free mass (FFM) (16), fat mass (FM) (5, 13, 16, 24, 25, 27, 28, 34), epididymal white adipose tissue (18), blood pressure (18, 25, 33), fasting plasma glucose (20, 26, 27, 31, 33, 34, 39), insulin concentration (20, 26, 31, 33, 34), triglycerides (5, 20, 27, 34), cholesterol (5, 20, 34, 38), LDL (5, 20, 24, 25, 38), HDL (20, 38), serum glycosylated hemoglobin (20, 31, 33), NEFA (27, 31, 33, 34, 39), etc. Additionally, inflammatory markers as tumor necrosis factor (TNF-α) (3, 13, 16, 25, 26, 28, 31, 33, 38, 39), interleukin 1β (26, 33, 39), IL-6 (13, 16, 24, 25, 27, 28, 33, 38, 39), IL-8 (20), activation of mitogen-activted protein kinases (MAPK) (26, 27, 31, 34), C-reactive protein content (CRP) (5, 16, 24, 25, 31, 38), monocyte chemo attractant protein-1 (MCP-1) (3, 13, 26, 28, 38) and activation of NF-κB (20, 31, 34, 38, 39) were also assessed.

Synthesis of main results

Human models

Previous to exercise interventions, upregulation of TLR4 mRNA and protein expression in mononuclear cells of type 2 diabetic patients has been reported (20). Similarly, higher basal TLR4 protein content in PBMC has also been described in obese diabetic sedentary subjects (31). In trained subjects, the pre-intervention TLR2, 4 and 7 mRNA levels were the same for obese runners as lean elite and non-elite marathon athletes (25).

Regarding the effects of long term interventions, twelve weeks of combined endurance and resistance exercises elicited significant reductions in TLR4 mRNA levels (Lambert 2008) and both mRNA and protein expressions (Liu 2015). These combining interventions produced larger decrease in TLR4 expression in mononuclear cells (20) and vastus lateralis (16) than drug and diet control therapies. In a third study, 10mg/d of rosuvastatin, was administered for 20 weeks, alone or in combination with endurance and resistance exercises (5). The sedentary group showed a higher TLR4 expression on CD14+ monocyte surface after rosuvastatin chronic intake compared to pre- ingestion values. Moreover, there were no significant differences in TLR4 surface expression between physically active control subjects and those combining rosuvastatin treatment and exercise training over the course of the study (pre- vs. mid- vs. post-time measurements) (5). Endurance training in marathon runners significantly increased TLR2 mRNA levels in dendritic cells of non-elite runners in comparison to their basal pre-intervention values, whilst TLR4 and TLR7 mRNA levels were up-regulated in obese, non-elite and elite runners (25). Long-term resistance training did not change TLR4 mRNA level in whole blood samples in obese post-menopausal women, compared with sedentary controls (28).

Short-term endurance protocols such as 15 days of interval training did not induce changes in TLR4 or TLR2 protein con-

tent in peripheral blood mononuclear cells in obese, diabetic or lean participants, when compared with their baseline values (31). High interval training and continuous aerobic training protocols showed different results depending on the blood cell analyzed (33). TLR4 surface expression was reduced on lymphocytes and CD14+ monocytes vs. pre-exercise expression, with no significant differences between interventions; while the surface expression in CD15+ neutrophils was only reduced by continuous exercise. Surface TLR2 lymphocyte expression decreased with either interval or continuous training compared with pre-exercise values; although it was not the case for monocytes and neutrophils (33).

Immediately after a marathon, in peripheral blood mononuclear cells, TLR2 mRNA levels remained unchanged when compared to pre-exercise levels in the three included groups: elite, lean non-elite and obese runners (24). TLR4 mRNA was down-regulated in lean non-elite only and TLR7 mRNA was reduced in all groups. Twenty-four hours after the race, TLR2 mRNA level was still unchanged, whilst TLR4 and TLR7 mRNA levels increased in all groups compared to baseline. TLR2-4-7 protein expression was only analyzed in lean non-elite runners and the only difference found was a decrease in TLR7 24h after marathon vs. pre-exercise values (24).

Animal models

A high fat diet resulted in an increase in TLR4 and MyD88 interaction in white adipose tissue when compared with standard chow (26). Furthermore, an increased TLR4 expression was reported in monocytes, adipose tissue, soleus, ventricle muscle, vascular tissue and bone derived macrophages of obese animals compared to their respective controls (7, 13, 18, 27, 34, 38, 39).

An endurance training of 6 weeks consisting in running on a treadmill resulted in less TLR4 cell surface expression on monocytes of animals consuming a high fat diet vs. their sedentary controls (7). Twenty-week old mice were fed with a high fat diet for 50 weeks. When reaching 70 weeks, the mice started an eight-week intervention consisting in 24h access to voluntary wheel running, coupled to low fat diet. This intervention produced lower TLR2 cell surface expression on monocytes than mice receiving a normal diet, sedentary mice or mice subjected to 60 min of forced running (3). Similarly, at the end of the intervention, voluntary exercised mice had the lowest TLR4 expression at monocyte surface compared to the 3 other groups (3). Protocols of similar duration also induced decreased mRNA and protein levels of TLR 2-4 in the soleus and ventricle muscles (18) and reduced mRNA and protein levels of TLR4 in liver, skeletal muscle and adipose tissue (27) of obese exercising compared to obese non exercising animals.

Long-term endurance training reduced TLR4 mRNA levels in obese animals compared with sedentary obese controls. Similarly, active or sedentary animals fed with standard chow did not increase TLR4 mRNA levels, thus these effects seemed to be additive as assessed by the interaction exercise x diet (13). Moreover, the reduction of TLR4 surface expression in obese animals after long term exercise interventions has been also related to modification on vascular miRNA-146A levels, in which its increment reduces chronic inflammation (38).

Compared to lean animals, TLR2 mRNA was lower, whilst TLR4 mRNA levels were higher, in macrophages of mice subjected to high fat diet (39). A short duration endurance training was not able to reverse those changes (39). Similar results were obtained while TLR2 mRNA decrease was induced by Porphyromonas gingivalis infection in obese mice (39). TLR4 protein expression in liver, adipose tissue and skeletal muscle remained unchanged in comparison to pre-exercise values in obese animals submitted to two acute bouts of moderate intensity exercise, whilst TLR4 mRNA expression was decreased, but in skeletal muscle only (27). After the same protocol, a decreased TLR4/MyD88 interaction has been reported in adipose tissue (26). Finally, TLR4 expression in the hypothalamus of obese rats remained constant after a single bout of treadmill running compared to pre-exercise values (34).

DISCUSSION

The findings of the present systematic review revealed a variety of responses of TLR mRNA, protein and cell surface receptor expression following exercise in humans or animals with obesity/MetS. The animal studies showed a marked tendency towards TLR2 and 4 mRNA, protein down-regulation and a reduction in TLR4/MyD88 interaction after endurance training. In humans, evidence is not sufficiently robust to determine the regulation of TLR expression after chronic or acute exercise. Multiple interventions types were conducted including endurance, resistance or a combination of both under diverse durations, frequencies, intensities, volumes or modes. Long-term endurance training for a marathon increased TLR mRNA levels according to the activity level and body composition of the athletes (25), which was also reflected after the marathon event (24). Furthermore chronic interventions of combined endurance and resistance exercises resulted in reductions of TLR4 mRNA and protein levels (16, 20), whereas no differences in TLR4 surface expression were observed when chronic endurance and resistance training were also combined with rosuvastatin intake(5). Moreover, long-term resistance protocol did not induce different TL4 mRNA expression from sedentary conditions (28). Short-term interventions elicited conflicting results, while in one study TLR2 and 4 protein content remained constant after interval training (31), another study performing either interval or continuous exercise, resulted in a different TLR surface expression depending on the blood cell analyzed (33).

Nevertheless, the baseline TLR mRNA and surface protein expression on mononuclear cells was consistently high in the obese and type 2 diabetic compared to healthy sedentary subjects. Interestingly, in obese physically active subjects the expression of TLR2, 4 and 7 mRNA expressions did not differ from the lean athletes, which was related to a lower pro-inflammatory state compared to obese sedentary subjects. Those general outcomes may be influenced by important factors that will be discussed below.

Intervention heterogeneity

There is a considerable heterogeneity within the human interventions. Differences in endurance protocols either by longer

(5, 16, 20, 25) or shorter duration (Robins 2015, Reyna 2013), lead to variances in the amount of exercise performed, similarly with the variety given by the type of endurance exercise used as continuous or intermittent (31, 33). Meanwhile, some training protocols were also accompanied with resistance protocols (5, 16, 20). Short term protocols, completed in 2 weeks (31, 33), elicited conflicting results that could have been influenced by differences in intensity and training mode where continuous exercises could have greater impact than intermittent protocols (33). In addition to the previous variations, acute strenuous endurance exercise (24) and sole resistance interventions were also executed (28) and could have been influenced by the training status of the subjects. After a marathon, the responses of TLR expression in peripheral blood cells were influenced by the acute intervention itself (24) and probably also by the chronic long-term training needed to complete such as strenuous exercise (25). The same holds true for acute resistance exercise (28) and the influence of training background (5, 16, 20) if not controlled. Lastly, sole resistance training failed to induce changes of TLR expression in whole blood and sub-cutaneous adipose tissue (28).

The studies conducted in animals used endurance training protocols that globally resulted in TLR down-regulation (3, 7, 13, 18, 26, 34), whereas acute interventions (26, 34) tended not to modify TLR expression. In addition to duration, other aspects may influence the outcomes between the selected studies, such as: switches in the dietary regimen (39), statin intake (e.g. rosuvastatin) (5) or induced infection (39). These factors lead to increased heterogeneity in the intervention protocols that could impact the immune response to exercise and should therefore be considered in the interpretation of the results.

Sample heterogeneity

The first aspect of heterogeneity was related to the participants' characteristics. The human studies included adults (24, 25, 31) and elderly subjects (5, 16, 20, 28, 33) of both genders or solely female/male groups. Similarly, the animal studies included male mice (3, 7, 13, 38, 39) or rats (18, 26, 27, 34) with specific genetic backgrounds and with an age varying between 4 to 66 weeks at the beginning of the study. Hence, age could have influenced the response of TLR to exercise but the literature reports conflicting results from comparisons between young and older participants (35) and animals (2, 30). Therefore, at this stage, there is no conclusive dependent effect of age on TLR levels and further research is needed.

Another relevant issue is the tissue analyzed and the level of expression of TLR, i.e. mRNA and/or protein. In human studies, PBMC (20, 24, 25, 31) and whole blood (5, 28, 33) are the samples used in the majority of the studies whereas only one reported analysis in skeletal muscle (16). Differences in TLR expression has been reported in different PBMC, i.e. monocytes, neutrophils and lymphocytes, emphasizing that the sole measure of monocytes is not representative of the total circulating immune cells (33). The measure of TLR in human skeletal muscle has been less explored while its detection might be relevant to reveal direct effects of exercise on TLR expression (16). In addition, the report of the specific localization of TLR appears to be important since it can be located on the cell surface or be internalized into endosomes (22). Within the selected studies, only two used color flow cytometry and could clearly report the surface localization (5, 33). Although specific to the type of TLR tested (4), knowing the localization in more studies would improve the thoroughness of the methodology in the field and therefore contribute to a better understanding of the regulation of TLR and of the immune system in general.

Methodological quality

Regarding the quality of the selected studies, the majority was of high quality, a few had moderate and only one had a low score. The above indicates a proper reporting of methodological aspects that leads to a better understanding and reliability of the outcomes of the studies. However, there were some items that were not optimally described and should be considered for further research. Half of the human studies did not fully describe the participants' characteristics or the selection procedure, which renders difficult the precise characterization of the population and the comparison between studies. Another inconsistency was found in the statistics used in both human and animal studies, in which parametric methods were used without reporting normality. Following the arguments of Weissgerber et al (36), parametric methods are appropriate only if symmetrical distribution was reported. An additional aspect missed in the vast majority of the studies was the report of blinding the examiner from participants' characteristics, which is necessary to ensure a suitable methodology. Based on the Gold Standard Publication Checklist to improve the Quality of Animal studies (10), there was an overall adequate report in the animals studies. However, in a few studies, essential information like weight at the start of the experiment and/or description of sample size calculation was not available. It was, therefore not possible to determine whether the amount of animals used reached the minimum required for the main outcome.

Clinical perspective

Obesity and MetS are characterized by a low grade inflammation state, associated with endocrine and metabolic disorders (14). This low grade inflammation state increases the risk of death and co-morbidities which are a major public health concern. Chronic exercise has the capacity to counteract, at least partially, a pro-inflammatory state through reduced activation of the pro- inflammatory pathway and cytokine production (29). Studies in the present review reported reduced activation of IKK and NF-κB signaling after exercise training (16, 20, 26, 27, 34, 38, 39). This reduction in NF-κB signaling was also accompanied by lower levels of TNF-α (5, 13, 25-28, 38, 39), CRP (5, 28, 38), MCP-1 (26, 39), IL1β (39) and IL8-33 (20). In addition, IL6 was reduced after endurance training protocols (16, 27, 38, 39), but an acute increment was also reported as a result of muscle contraction following resistance training (28) and acute aerobic bouts (Ropelle et al., 2010, Nickel et al., 2013). Furthermore, in both trained human and animal studies, a reduction of MAPK activation, i.e. ERK and JNK phosphorylation, was described (26, 27, 31), which reflects the influence of exercise on the downstream signals of the TLR pathway.

Insulin resistance is one of the major complications provoked by metabolic disorder while exercise acts as an immunomodulator that can delay its aggravation and the appearance of type 2 diabetes mellitus (17). Within the selected studies, various markers of insulin resistance were reduced after the interventions: fasting blood glucose (20, 26, 27, 33), postprandial blood glucose, postprandial plasma insulin and glycated hemoglobin (20). In terms of insulin sensitivity, Reyna et al reported an improvement of about 10% in obese and about 30% in type 2 diabetic subjects (31). Decreased serum insulin levels (26, 27) and restored insulin signaling, reflected by an increase in insulin-induced tyrosine phosphorylation of the insulin receptors (34), were also described.

Concerning body composition, lower BMI and body weight (3, 25, 31, 33, 34), decrease in waist circumference (25) and fat-free mass (16) were found after endurance training. Modifications of lipid metabolism were also reported. Leptin concentrations were significantly reduced after resistance training (28). It was also reported that the level of oxidatively modified low-density lipoproteins decreased after strenuous endurance training and that serum LPS levels decreased after either acute exercise or training in animals (27).

Limitations

Considering the analytical techniques and the way the main outcomes were reported, a meta-analysis was not feasible. Hence, a quantifiable comparison of TLR expression was out of our possibilities. Another aspect to be mentioned is the presence of a large clinical and methodological heterogeneity, which makes difficult to do conclusive interpretations. Additional interventions in some studies such as drug administration, or induced infections, may influence immune environment and could induce bias when looking at the effect of exercise on TLR expression.

CONCLUSIONS

The findings of the present systematic review based on 17 human and animal studies show that exercise elicits a variety of responses in TLR mRNA, protein and cell surface receptor expression. These results may have been influenced by the sample hereogeneity given by the participant's characteristics and by the different tissues analyzed. Although not all studies reported changes in TLR level, reduction in cytokine production and NF-κB/MAPK activation indicate the amelioration of the chronic pro-inflammatory state, which was also related to increased insulin sensitivity. The effects of exercise on TLR expression and downstream anti-inflammatory responses seem to be related to exercise type and duration, suggesting that diverse signaling cascades may possibly be stimulated depending on the exercise protocols. To get a better insight, further human research is needed using interventions on a longer term, even up to years. Additionnally, TLR expression should be reported at both mRNA and protein levels, analyzing tissues sensitive to insulin (i.e. skeletal muscle, liver and adipose tissue).

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