Metabolic signals and innate immune activation in obesity and exercise

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ABSTRACT

The combination of a sedentary lifestyle and excess energy intake has led to an increased prevalence of obesity which constitutes a major risk factor for several co-morbidities including type 2 diabetes and cardiovascular diseases. Intensive research during the last two decades has revealed that a characteristic feature of obesity linking it to insulin resistance is the presence of chronic low-grade inflammation being indicative of activation of the innate immune system. Recent evidence suggests that activation of the innate immune system in the course of obesity is mediated by metabolic signals, such as free fatty acids (FFAs), being elevated in many obese subjects, through activation of pattern recognition receptors thereby leading to stimulation of critical inflammatory signaling cascades, like IκBα kinase/nuclear factor-κB (IKK/NF-κB), endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) and NOD-like receptor P3 (NLRP3) inflammasome pathway, that interfere with insulin signaling. Exercise is one of the main prescribed interventions in obesity management improving insulin sensitivity and reducing obesity-induced chronic inflammation. This review summarizes current knowledge of the cellular recognition mechanisms for FFAs, the inflammatory signaling pathways triggered by excess FFAs in obesity and the counteractive effects of both acute and chronic exercise on obesity-induced activation of inflammatory signaling pathways. A deeper understanding of the effects of exercise on inflammatory signaling pathways in obesity is useful to optimize preventive and therapeutic strategies to combat the increasing incidence of obesity and its co-morbidities.

Key words: exercise, immune system, obesity, fatty acids, inflammation, adipose tissue

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1. INTRODUCTION

Excess energy intake and reduced energy expenditure have been recognized as the most important aetiologic factors for obesity indicating that obesity is largely preventable by an appropriate lifestyle. Despite this knowledge, obesity is one of the major public health concerns with prevalence rates dramatically rising worldwide. According to prospective estimations, about 2.3 billion adults in the world will be overweight or obese by the year 2015 (104). This fact poses significant problems for healthcare systems, since obesity constitutes a risk factor for a large number of health problems, ranging from merely the physical burden of the excess adipose tissue itself (e.g. joint pain, back pain, dyspnoea) to serious endocrine and metabolic disturbances, such as type 2 diabetes (T2D), cardiovascular disease, hepatic steatosis, airway disease, biliary diseases and certain cancers (104). Aggravating this situation is the fact that the obesity-related disturbances are linked to reduced life expectancy and premature death. Thus, understanding the biological basis for the development of obesity-related disturbances is an important need.

Obesity is characterized by a pathologic expansion of adipose tissue (AT) that is caused mainly by an enlargement of pre-existing fully differentiated adipocytes due to the storage of excess energy as fat (87). AT expansion through adipocyte enlargement is critical, because it leads to insulin resistance (IR) (54, 91, 103); a condition of reduced glucose utilization by insulin-sensitive tissues due to an impaired insulin action. IR is thought to be a key driver for developing obesity-related endocrine and metabolic disturbances (8, 39, 45, 76). Intensive research during the last two decades has revealed that a characteristic feature of obesity linking it to IR is the presence of chronic low-grade inflammation, which develops locally in the expanding AT, but becomes systemic through the release of numerous pro-inflammatory mediators including cytokines into the blood stream (24-26, 97). As the metabolic surplus (excess nutrients and energy) is thought to be the initial signal for the inflammatory response, the chronic inflammation associated with obesity is referred also to as metaflammation (metabolically triggered inflammation) (28). The AT-derived pro-inflammatory mediators are initially secreted by the enlarged adipocytes, but with increasing AT expansion also by macrophages infiltrating the AT (29, 100). Interestingly, diet induced obesity was found to cause a phenotypic shift of AT macrophages from the M2 polarization state, which exhibits secretion of anti-inflammatory cytokines, to the M1 polarization state, which produces large amounts of pro-inflammatory cytokines, (53). Apart from

macrophages, other immune cells such as mast cells and natural killer T cells are known to increase in AT in obesity and contribute to the pro-inflammatory milieu (51, 69). Moreover, the ratio of $CD8^+$ to $CD4^+$ T cells has been found to increase in the obese AT, whereas the number of immunosuppressive $CD4^+$ regulatory T cells, which are known to secrete anti-inflammatory cytokines that inhibit macrophage migration, in obese AT decreases (17, 66, 105). This clearly indicates that obesity is associated with the activation of the innate immune system, a system that is important to respond to microbial stimuli, such as bacterial, viral and fungal infections, by coordinating an inflammatory reaction and subsequent tissue repair. Detection of microbial stimuli by the innate immune system occurs by a set of pattern recognition receptors (PRRs), which have evolved in mammals to sense and trigger a response to common microbial structures. Considerable evidence exists that many PRRs act also as sensors of metabolic signals, such as free fatty acids (FFAs), and upon activation critical inflammatory signaling cascades, such as the IκBα kinase/nuclear factor-κB (IKK/NF-κB) pathway, endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) pathway and the NOD-like receptor P3 (NLRP3) inflammasome pathway are stimulated providing a plausible explanation that an inflammatory response is induced during metabolic surplus.

This review summarizes current knowledge of the cellular recognition mechanisms for FFAs, which are chronically elevated in the circulation of obese subjects (35), and critical intracellular inflammatory signaling pathways triggered by excess FFAs in obesity. The review also examines the beneficial effects of exercise, which is one of the main prescribed interventions in obesity management improving insulin sensitivity and reducing obesity-induced chronic inflammation.

2. RECOGNITION MECHANISMS FOR FFAs

FFAs are known to interact with several receptors, such as PRRs, like Toll-like receptors (TLRs) and nucleotide-binding, oligomerization domain containing receptors [NOD-like receptors (NLRs)], and FFA receptors (FFARs). Interestingly, despite being essential components of the innate immune system, PRRs are present in both immune cells and metabolically active tissue cells including hepatocytes, myofibrils, and adipocytes to initiate inflammatory signaling cascades (64).

2.1 PATTERN RECOGNITION RECEPTORS (PRRs)

The PRRs have been initially described to be involved in the response to microbial attack through sensing/detecting unique pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide, microbial peptides and proteins (muramyl dipeptides, flagellin), and double-stranded ribonucleic acids (dsRNA), and triggering a subsequent immune response. More recently, it has become apparent that PRRs not only recognize microbial signals but also mediate immune responses to endogenous "danger" signals [danger-associated molecular patterns (DAMPs)], including those arising from metabolic disturbances, like saturated fatty acids, ceramides, cholesterol crystals, monosodium urate crystals, amyloid beta, and extracellular ATP (64, 81).

TOLL-LIKE RECEPTORS (TLRs)

One class of PRRs are the TLRs, which are mammalian homologues to the Toll gene in drosophila (the fruit fly), where it encodes a receptor for host defence against microbial infections. The TLRs, of which more than 10 members exist in humans and mice, sense PAMPs via their extracellular leucine-rich repeat domain leading to TLR dimerization through adaptor protein recruitment to their cytoplasmic Toll/IL-1 (TIR) domain, which activates downstream signaling adaptor proteins including MyD88, IRAKs and TRAF6. At least two TLRs, namely TLR4, which is best-known to be activated by the fatty acid residue in the lipid A component of LPS, and TLR2 have been shown to be activated by saturated FFAs (16, 84), and to mediate FFA-induced impairment of insulin sensitivity through negatively interfering with insulin signaling (84). In addition, FFAs were found to exacerbate pro-inflammatory cytokine secretion from M1 macrophages through activation of TLR4, which is highly expressed by pro-inflammatory M1 macrophages and thus used as a marker for M1 macrophages (29), in genetically and diet-induced obese mice thereby promoting AT inflammation (65). A key role of TLR4 for developing IR was evidenced from the observation that TLR4 knockout mice are protected from IR when fed a high-fat diet (HFD) (74, 96). Likewise, knockout of TLR2 was found to provide protection from HFD-induced IR and to reduce AT macrophage accumulation and AT inflammation (10, 14, 21).

NOD-LIKE RECEPTORS (NLRs)

The cytoplasmic NLRs, of which 22 members are known in humans (81) are classified into five subfamilies: NLRA, NLRB, NLRC, NLRP and NLRX. All NLRs contain a central NACHT domain, which is responsible for oligomerization, and a C-terminal leucine-rich repeat domain, which facilitates sensing of PAMPs and DAMPs. The NLR subfamilies differ in their N-terminal effector domains, which ascribe unique functional properties to the NLRs. Members of the NLRP family, which contain a pyrin domain (PYD) as effector domain for downstream signaling, are best known for their role in inducing the formation of the multi-protein inflammatory complex, called "inflammasome", in response to stress signals (56). The NLRP3 inflammasome is the most commonly studied and has attracted great attention as the protagonist in obesity-associated inflammation and IR (63) due to its ability to be activated by saturated fatty acids, ceramide and reactive oxygen species (ROS) and to negatively regulate insulin receptor signaling (52, 101). Upon activation by PAMPs and DAMPs, the NLRP3 inflammasome is formed through recruiting the apoptosis-associated speck-like protein (ASC), containing an N-terminal PYD and a C-terminal caspase activation and recruitment domain (CARD), and the zymogen pro-caspase-1 to NLRP3, which is a prerequisite to induce caspase-1 activation. The activated caspase-1 subsequently cleaves the inactive cytokine precursors pro-IL-1β and pro-IL-18 into their biologically active forms, thereby inducing pro-inflammatory cell death, called pyroptosis. The mature IL-1β, in particular, is critical with regard to the induction of IR because it causes cell death of pancreatic β-cells, inhibits AKT phosphorylation coincident with serine phosphorylation of IRS-1, and, interestingly, mediates inter-organ cross-talk between adipocytes and the liver, promoting systemic inflam-

mation and lipotoxicity (30, 47, 67). The critical role of NLRP3 in obesity-induced AT inflammation and IR is shown by the fact that NLRP3 knockout mice are resistant to IR and exhibit reduced AT inflammation when kept on a HFD (92, 98, 101). In humans, weight loss in obese T2D subjects leads to reduced AT expression of NLRP3 and AT inflammation and improved insulin sensitivity (98).

2.2 FREE FATTY ACID RECEPTORS (FFARs)

The FFARs are G-protein coupled receptors (GPRs) that are widely expressed in tissues and activated by either medium and long-chain fatty acids (FFA1 and FFA4 also known as GPR40 and GPR120, respectively) or short chain fatty acids (FFA2, FFA3). FFA1 in particular is considered to be an important key that links chronically elevated FFAs in obesity to IR and T2D. FFA1 was found to mediate the lipotoxic effects of saturated FFA in pancreatic β-cells through disturbing cytosolic calcium ion (Ca^{2+}) homeostasis, amplifying glucose-stimulated insulin secretion, thereby causing β-cell dysfunction and β-cell apoptosis (1, 83). In contrast, inhibition of GPR40 protected MIN6 β-cells from palmitate-induced apoptosis (108). In line with this, loss of FFA1/GPR40 was found to protect mice from obesity-induced hyperinsulinaemia, hepatic steatosis, and glucose intolerance (89).

3. INFLAMMATORY SIGNALING PATHWAYS ACTIVATED BY FFAs IN OBESITY

3.1 IKK/NF-KB PATHWAY

The IKK/NF-κB pathway is a well-known inflammatory signaling pathway that is activated upon stimulation of the cell by various agents, including cytokines, growth factors, ROS and microbial components like LPS. All these agents trigger different upstream signaling cascades that converge in the activation of the IKK enzyme complex leading to phosphorylation of the NF-κB inhibitor IκBα and its subsequent degradation via the proteasome. This causes the release of the nuclear localization sequence of NF-κB and its translocation into the nucleus where it stimulates transcription of genes involved in inflammation including pro-inflammatory cytokines, chemokines, adhesion molecules and many others. In addition, IKK activation impairs insulin signaling, thereby inducing IR.

Like LPS, saturated FFAs, such as palmitic acid, are reported to cause IKK activation and IκBα phosphorylation through the activation of TLR4 in different cell types including adipocytes, vascular cells, and macrophages (75, 84). Conversely, NF-κB target genes are not induced by FFAs in TLR4 knockdown adipocytes and obese mice lacking TLR4 are partially protected from HFD-induced inflammatory gene expression and IR (84). Likewise, mice with a spontaneous loss-of-function mutation in TLR4 are protected from IR, weight gain and adiposity when kept on a HFD (96).

3.2 ENDOPLASMIC RETICULUM STRESS-INDUCED UNFOLDED PROTEIN RESPONSE

The ER is a dynamic continuous membrane-enclosed organelle with distribution throughout the cytoplasm and has important functions in protein biosynthesis, folding and trafficking and Ca^{2+} storage and signaling. Consequently, when the protein synthetic and folding capacity and Ca^{2+} homeostasis of the ER are perturbed - a condition referred as ER stress - a complex cascade of cytoplasmic and nuclear signaling pathways is activated, which is collectively called the UPR and which is comprised of the inositol requiring 1 (IRE1), the PKR-like ER kinase (PERK) and the activating factor 6 (ATF6) pathway. Initially, the UPR aims to reestablish ER homeostasis through inhibition of protein translation to decrease ER load, transcriptional activation of chaperone genes to increase the ER folding capacity, and activation of the ER-associated degradation (ERAD) machinery to clear misfolded proteins (7). In addition, the UPR pathway enhances inflammation through PERK-eIF2α-dependent translational suppression of IκB leading to nuclear translocation of NF-κB. Moreover, sinceIRE1 and PERK cause activation of JNK and IKKβ signaling pathways, ER stress-induced UPR also mediates IR through impairing insulin signaling. Ultimately, the ER stress-induced UPR can trigger cell death by the induction of apoptosis, if ER stress cannot be resolved (3, 80).

Convincing evidence demonstrates that ER stress is present in tissues including AT, liver, and skeletal muscle, of obese subjects and obese animals (19, 27, 38, 72) and it has been proposed that ER stress links obesity, IR and T2D (72). Conversely, weight loss decreases ER stress coincident with improved insulin signaling in tissues of obese subjects (19). In addition, several studies have shown that saturated FFAs, which are present at elevated levels in obesity, cause ER stress in different cell types including liver cells, pancreatic β-cells and adipocytes (13, 31, 40, 99) indicating that FFAs are potential mediators for the induction of ER stress in obesity. Evidence has been provided that ER stress can be induced by FFAs via both FFARs and TLRs; for example, while inhibition of FFA1/GPR40 was found to protect MIN6 cells from palmitate-induced ER stress (108), TLR4 deficiency was reported to prevent HFD-induced ER stress and IR in the main organs for glucose and lipid metabolism (skeletal muscle, liver, and AT) in mice (74).

3.3 NLRP3 INFLAMMASOME PATHWAY

Formation of the cytosolic NLRP3 inflammasome complex, consisting of NLRP3, caspase-1 and ASC, involves a two-step process: 1) In the priming step, a first hit (signal 1) causes transcriptional induction of inflammasome components, including NLRP3 and pro-IL-1β and pro-IL-18, via TLR-mediated activation of NF-κB. This induction is necessary because basal expression of NLRP3 in resting cells is insufficient for effective inflammasome activation, with the exception of human blood monocytes and dendritic cells which have constitutive NLRP3 inflammasome activity (20, 86). 2) In the activation step, a second hit (signal 2) promotes the NLRPs to undergo homotypic oligomerization and assemble the active inflammasome capable of converting the cytokine precursors into active IL-1β and IL-18. Although the precise molecular mechanisms involved in the activation of the NLRP3 inflammasome in response to PAMPs including bacterial pore-forming toxins (nigericin, listeriolysin O, pneumolysin, α-haemolysin) and DAMPs remain to be elucidated, it has been suggested that potassium ion (K^+) efflux is one common cellular response to diverse stimuli triggering

inflammasome activation (81). For instance, crystals (e.g., urate) and particulate DAMPs, entering the cell via endocytosis, and pore-forming toxins activate the NLRP3 inflammasome via facilitating K^+ efflux (23, 55, 58, 61). Extracellular ATP released from dying or damaged cells activates the NLRP3 inflammasome through binding the purinergic receptor P2X7, which induces opening of pannexin-1 channels thus resulting in K^+ efflux and influx of any DAMPs and PAMPs present in the extracellular space (2, 18, 34). Besides triggering K^+ efflux, recent studies suggested that Ca^{2+} signaling and mitochondrial destabilization plays a critical role for NLRP3 inflammasome activation (22, 48, 79). For instance, different crystals (e.g., urate, silica, cholesterol) trigger Ca^{2+} influx through opening TRPM2 channels (62, 111). As a consequence, Ca^{2+} accumulates in the cytosol causing mitochondrial destabilization or dysfunction and release of mitochondrion(mt)-associated ligands, like mtDNA and cardiolipin, and mtROS, all of which activate the NLRP3 inflammasome. Apart from stimulating extracellular Ca^{2+} influx, elevated Ca^{2+} transfer from the ER to mitochondria at the ER-mitochondria contact sites, the mitochondrial-associated ER membranes,has been shown also to trigger the cascade of mitochondrial destabilization, release of mitochondrion-associated ligands and NLRP3 inflammasome activation (60).

Saturated FFAs can act as both primers and activators in the activation of the NLRP3 inflammasome. Mechanistic studies have demonstrated that saturated FFAs are particularly potent signals to induce the priming step of NLPR3 inflammasome activation through TLR2/4-dependent activation of NF-κB (65, 102). L´Homme et al. (50) demonstrated that both palmitic acid and stearic acid increase IL-1β release through NLRP3 inflammasome activation in LPS-primed human monocytes, in human monocyte-derived macrophages and in THP-1 macrophages. Recently, it was also shown that the saturated FFA palmitic acid induces NLRP3 inflammasome activation via induction of ER stress, which primes cells for pro-IL-1β production via NF-κB and promotes IL-1β secretion (44). In addition, Kim et al. (44) found that ER stress-induced ROS production activates the NLRP3 inflammasome through binding of the ROS-sensitive NLRP3 ligand thioredoxin-interacting protein (TXNIP), resulting in IL-1β cleavage and secretion. These findings indicate that FFAs through inducing ER stress act as primers and activators of the NLRP3 inflammasome.

4. EFFECT OF EXERCISE ON OBESITY-INDUCED ACTIVATION OF INFLAMMATORY SIGNALING PATHWAYS

Exercise is a reasonable approach to attenuate diet-induced weight gain by increasing energy expenditure and counteracting a positive energy balance (57, 73), thereby, providing several benefits for skeletal muscle function including increased insulin sensitivity, stimulated utilization of metabolic substrates, and even improved protection against oxidative insults. In the following sections evidence is provided that these beneficial effects of both acute and chronic exercise are mediated on the molecular level by an inhibition on obesity-induced activation of inflammatory signaling pathways being responsible for attenuating obesity-induced metaflammation.

4.1 EFFECTS OF EXERCISE ON THE IKK/NF-KB PATHWAY

It is well established that the IKK/NF-κB pathway is activated in tissues of obese subjects and obese animals (70, 93), and that activation of this pro-inflammatory signaling pathway is a key pathogenic mechanism responsible for inhibition of insulin signaling and induction of IR. Since it is also well known that exercise reduces obesity-induced IR (77, 94), it is likely that exercise prevents the development of obesity-induced IR through inhibiting the NF-κB pathway in insulin-dependent tissues. Indeed, several studies have documented the potential of exercise training to attenuate obesity-induced activation of the IKK/NF-κB pathway. For instance, Medeiros et al. (59) demonstrated that 12 weeks of endurance exercise training (swimming at 32°C water temperature, 1h/day with 5 % overload of the body weight, 5 days/week) inhibits the NF-κB pathway and increases the activity of the mTOR/p70S6k pathway of insulin-dependent protein synthesis thereby reducing IR in the cardiac tissue of diet-induced obese rats. In addition, Da Luz et al. (9) demonstrated that 8 weeks of endurance exercise training (swimming at 32°C water temperature, 1 h/day with 5 % overload of the body weight, 5 days/week) reduces activation of the NF-κB pathway in AT and liver via ameliorating ER stress along with improving insulin sensitivity in diet-induced obese rats. Moreover, Oliveira et al. (70) reported that both acute (two 3 h swimming sessions at 34°C water temperature, separated by a 45 min rest period, with 5 % overload of the body weight) and chronic exercise (same conditions as for acute exercise, 1 h/day, 5 days/week, 8 weeks) inhibits the IKKβ/NF-κB pathway and, in parallel, improves insulin signaling in tissues (AT, skeletal muscle, liver) in diet-induced obese rats. Also in humans, an inhibitory effect of 8 weeks of aerobic exercise training on a stationary bicycle (four times/week, exercise intensity, duration, and frequency were progressively increased to 70 % of VO_2max for 45 min during the 8-week exercise program) on NF-κB pathway in vastus lateralis muscle was found in T2DM subjects (88).

With regard to the mechanism of inhibition of obesity-induced NF-κB activation by exercise, Oliveira et al. (70) demonstrated in their study that both acute and chronic swimming exercise reverses obesity-induced activation of TLR4, which is an activator of both IKKβ and JNK in tissues of diet-induced obese rats. This suggests that the effect of exercise involves suppression of FFA-induced activation of TLR4 signaling, because TLR4 is activated by FFAs whose levels are typically elevated in obese subjects. Reduced FFA-mediated activation of TLR4 signaling by chronic exercise is probably also the result of decreasing the expression of TLR4 in AT. This was observed in HFD-induced obese mice in response to a 16-weeks endurance exercise protocol on a motorized treadmill (60 min/day, 5 times/week) (37). The reduced TLR4 expression in AT is likely the consequence of exercise-induced suppression of M1 macrophage infiltration, which express high levels of TLR4 (29), and/or phenotypic switching from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages (36, 37). TLR4 plays a key role for activating the pro-inflammatory NF-κB pathway and develop-

ing IR during obesity as evidenced from the finding that TLR4 knockout mice are protected from IR and induction of pro-inflammatory gene expression when fed a HFD (74, 96). In addition, activation of NF-κB by FFAs is prevented in TLR4 knockdown adipocytes (84). Given that long-term exercise training on a stationary bicycle in humans (exercise intensity of 50-70 % of the target heart rate, 30 min/day, 5 times/week for 12 weeks) causes a reduction of body fat along with a decrease of resting plasma FFA levels (41), the reduced activation of NF-κB in tissues might be simply explained by a reduced TLR4 activation and expression due to decreased plasma FFA levels (70, 71, 110). In contrast, it is well-established that a prolonged acute exercise bout increases circulating FFA levels due to increasing mobilization from AT in order to provide energy substrates for contracting skeletal muscle. In line with this, an acute exhaustive exercise bout (treadmill running at 70% VO_2max for 50 min and then running at an elevated rate that increased by 1 m/min until exhaustion, mean exhaustion time: 61.98 ± 2.24min) was found to activate TLR4 signaling and the NF-κB pathway in AT of healthy, non-obese rats (78). However, despite their well known role in impairment of insulin action, it is expected that TLR activation during acute exercise does not impair insulin sensitivity. This assumption is supported by a study using TLR2 and TLR4 knockout mice, in which the increase of circulating FFAs during acute exercise was stronger than in wild-type mice. These data indicated a role of these proteins in metabolic regulation or repartition of energy substrates during acute exercise (109).

Noteworthy, it was also found that suppression of TLR4 signaling in tissues of HFD-induced obese rats in response to the abovementioned chronic swimming exercise protocol is accompanied by a decrease of serum levels of LPS (70), which is a known activator of TLR4 signaling and, through this, of the IKK/NF-κB pathway. Elevated serum LPS levels have been observed following the intake of a HFD and are probably the result of an increased intestinal permeability for LPS (5). These findings indicate that the inhibitory effect of exercise on NF-κB activation in HFD-induced obese rats might be also due to attenuating LPS-induced TLR4 activation. In this context it is also interesting that a strenuous endurance exercise bout (marathon run) was followed by a slight LPS IgG activity indicating a mild endotoxaemia (4), which has been suggested to be the result of an increased intestinal permeability after exercise.

4.2 EFFECT OF EXERCISE ON THE ENDOPLASMIC RETICULUM STRESS-INDUCED UNFOLDED PROTEIN RESPONSE

It is well documented that exercise (acute and chronic) causes ER stress-induced UPR in skeletal muscle of lean, metabolically healthy humans and animals (42, 43, 68, 107). One important stimulus for ER stress-induced UPR is considered to be the elevated production of ROS during acute muscle contraction (43). In addition, altered Ca^{2+} dynamics and elevated protein synthesis in response to resistance exercise could also provoke the UPR (12). Moreover, mechanical stress and/or local metabolic changes in the muscle that are directly involved in exercise are thought to play a role in UPR activation (107). In line with these divergent ER stimuli, the stimulatory effect of exercise on ER stress-induced UPR pathway was evident in response to different kinds of exercise: A single bout of exhaustive exercise in muscles heavily activated during treadmill exercise (starting at a warm up speed of 5 m/min for 5 min, every subsequent 5 min, the speed increased by 5 m/min until mice were exhausted or a maximal speed of 25 m/min was reached) in mice (M. quadriceps and M. gastrocnemius but not in non-weight bearing back muscle M. erector spinae) (107); a long lasting running exercise (200 km run, 28 ± 2 h) in M. vastus lateralis in humans (42); a single unaccustomed resistance-exercise bout (leg press and knee-extension exercise) in M. vastus lateralis in humans (68). This indicates that both endurance and resistance exercise causes ER stress-induced UPR in skeletal muscle. Interestingly, acute exercise was found to activate specifically the ATF6/IRE1α pathway of the UPR, but not the PERK/eIF2α pathway, that attenuates protein synthesis, (43, 68), thereby promoting the production of certain chaperone proteins, likely as a consequence of the increased production of various proteins during the post-exercise period (68). Activation of the UPR in skeletal muscle by chronic exercise was found to be an important mechanism in the adaptation of skeletal muscle to exercise training (107), with the transcriptional co-activator PGC1α, which regulates several exercise-induced adaptations of skeletal muscle function (mitochondrial biogenesis and function, oxidative metabolism, fibre type switching, angiogenesis), being the mediator of the UPR in skeletal muscle in response to exercise (107). Regulation of the UPR by PGC1α during exercise was shown to involve direct co-activation of ATF6, which is one of the three proximal sensors of the UPR preferentially activating UPR target genes involved in the adaptation of cells to chronic ER stress (106). The essential role of PGC1α and the UPR sensor ATF6 for the adaptation to exercise was evident from the observations that 1) muscle-specific PGC1α knockout mice are defective in up-regulating ER chaperones and experience exacerbated ER stress after repeated exercise training, and 2) ATF6a knockout mice do not recover from muscle damage after exercise (107).

Only very few studies are available in the literature investigating the potential of exercise training to attenuate obesity-induced ER stress. For instance, Da Luz et al. (9) demonstrated that 8 weeks of endurance exercise training (swimming at 32°C water temperature, 1 h/day with 5 % overload of the body weight, 5 days/week) following a 2-month HFD feeding period reduced PERK and eIF2α phosphorylation, inhibited pro-inflammatory signaling pathways (JNK, IκB and NF-κB), and reversed IR in AT and liver of obese rats suggesting that endurance exercise training reduces obesity-induced ER stress in tissues. In contrast, Deldicque et al. (11) reported the opposite finding, namely, that 6 weeks of endurance exercise training (treadmill running at 75 % VO_2max, 30-60 min/day, 5 days/week) during HFD feeding even promoted the UPR induced by HFD feeding alone in two different muscles (M. soleus, M. tibialis), liver and pancreas, despite attenuating the pro-inflammatory state induced by HFD feeding (11). Based on these findings Deldicque et al. (11) postulated that exacerbation of the HFD-induced UPR by endurance exercise might be an adaptive protective mechanism to restore ER homeostasis and to protect against obesity-induced inflammation. The opposing outcomes of endurance exercise on obesity-induced ER stress may have different reasons: 1) The availability of FFAs, which are likely mediators for the induction of ER

stress in obesity via activating both FFARs and TLRs, may be influenced by the different exercise interventions. While exercise intensities of about 75 % VO_2max as applicable to the treadmill protocol increases mobilization of FFAs from AT, glucose is preferentially utilized during the swimming protocol. This is evidenced by the observation that blood concentrations of epinephrine and lactate of mice are markedly higher following a swimming test (at 22°C water temperature until exhaustion; mean swimming time: 36.7 ± 3.7 min) compared to a running test (at 80 % VO_2max until exhaustion; mean running time: 39.5 ± 5.0 min) (46). Thus, it is likely that endurance exercise training during parallel HFD feeding amplified the obesity-induced ER stress due to the great availability of FFAs, whereas the obesity-induced ER stress could be attenuated by swimming exercise with only less FFA mobilization. 2) The authors of the treadmill study proposed that the obese rats were accustomed to the HFD feeding and that a new homeostasis level was reached before the swimming exercise stress was applied, so that the beneficial effects of exercise could be exerted. This was not the case in the treadmill study as both HFD feeding and treadmill exercise started simultaneously, each stress probably exacerbating the other. Interestingly, Deldicque et al. (11) also found muscle type-specific differences in the UPR activation by exercise in obese rats as activation of the UPR was very strong in the tonically active M. soleus and less pronounced in the phasically active M. tibialis anterior. This has been explained by a greater susceptibility of oxidative fibres to HFD feeding, an additive effect of contractile activity and HFD feeding on ER stress, or increased utilization of FFAs in M. soleus due to its higher metabolic requirements (11).

4.3 EFFECT OF EXERCISE ON THE NLRP3 INFLAMMASOME PATHWAY

To date, no published studies are available exploring the direct effect of exercise on obesity-induced activation of the NLRP3 inflammasome pathway. Unpublished data of our own group demonstrate that both endurance (treadmill running at 80 % VO_2max, 30 min/day, 5 times/week for 10 weeks) and resistance exercise (holding on a metal mesh placed in a vertical position, three 3 min bouts, with 1 min break between each bout, 5 times/week for 10 weeks) in HFD-induced obese mice decreases the mRNA level of NLRP3 in AT, which provides the first direct evidence for inhibition of obesity-induced NLRP3 activation by exercise. Interestingly, our data show that the decreased mRNA level of NLRP3 in AT of trained obese mice is accompanied by reduced plasma levels of IL-18, to which AT is considered a major contributor (15, 82, 85) and whose maturation and secretion is mediated by the NLRP3 inflammasome. Thus,

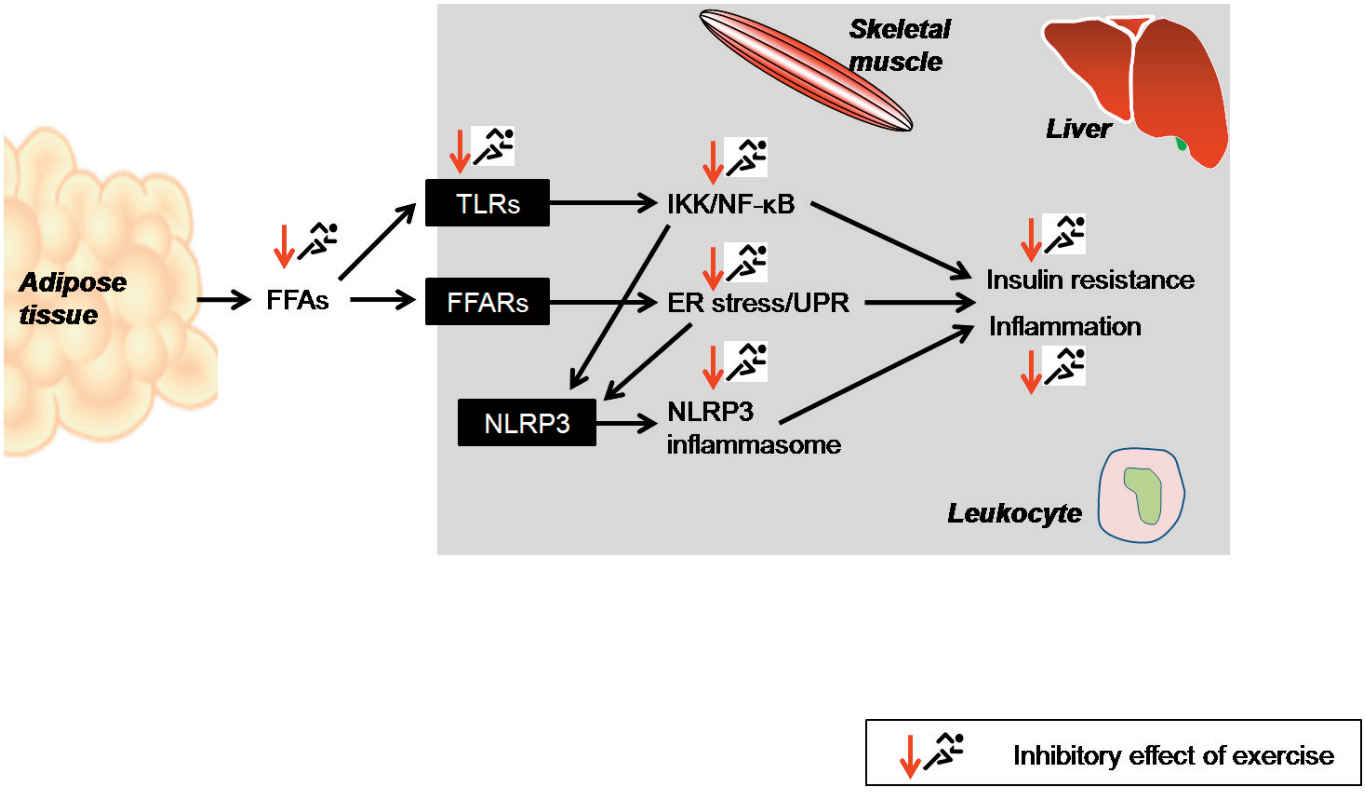

Figure 1
Schematic summary of the effects of exercise on obesity-induced activation of inflammatory signaling pathways. In the course of obesity, elevated levels of free fatty acids (FFA) led to the activation of critical inflammatory signaling pathways, like IκBα kinase/nuclear factor-κB (IKK/NF-κB) and endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) via activating pattern recognition receptors such as Toll-like receptors (TLRs) and free fatty acid receptors (FFARs). In addition, FFAs are potent signals to induce the priming step of NOD-like receptor P3 (NLRP3) inflammasome activation through TLR-dependent activation of NF-κB and via induction of ER stress. Through activation of these criticial inflammatory signaling pathways inflammation and insulin resistance is induced in metabolically active and immune cells. Exercise reduces chronic low-grade inflammation and insulin resistance through increasing utilization of FFAs, lowering expression of TLRs and attenuating activation of IKK/NF-κB, ER stress-induced UPR and NLRP3 inflammasome.

alterations in the plasma IL-18 level can be used to evaluate indirectly changes in the activity of the NLRP3 inflammasome pathway. Interestingly, a large number of human studies have reported that exercise reduces the plasma levels of IL-18 in obese subjects providing at least indirect evidence for inhibition of the NLRP3 pathway by exercise. For instance, Stensvold et al. (90) showed that the serum level of IL-18 was reduced by 43 % in response to 12 weeks of aerobic interval training (three times/week) in men and women with metabolic syndrome. Likewise, in overweight individuals with T2DM a 6-month aerobic exercise training program (four times/week, 45-60 min/session, 50-85 % VO_2max) *per se* (without affecting body weight) resulted in a significant reduction of the plasma IL-18 level (32, 33). Troseid et al. (95) found that the serum level of IL-18 was reduced by 12 weeks of exercise training in subjects with metabolic syndrome. Furthermore, 8 weeks of high-intensity exercise training on a rowing ergometer (three times/week, 30 min/session, $\geq$ 70 % VO_2max) decreased IL-18 mRNA level in abdominal AT and numerically lowered plasma IL-18 concentration in obese men and women (49). Only in one study by Christiansen et al. (6), a 12-week aerobic exercise training program (three times/week, 60-75 min/session), failed to reduce the plasma level of IL-18 in obese men and women. The lack of an exercise effect in this study may be explained by the relatively moderate exercise intensity.

Currently, it is not known whether exercise exerts its predominantly inhibitory action on NLRP3 activation in obese subjects in the priming step, which involves transcriptional induction of inflammasome components via TLR-mediated activation of NF-κB, in the activation step or in both steps of the NLRP3 activation process. Given that exercise was found to strongly reverse the activation of TLR4 signaling along with reducing IKKβ phosphorylation in tissues (AT, skeletal muscle, and liver) of diet-induced obese rats (70), suggests that exercise inhibits the NLRP3 inflammasome pathway in the priming step. Important primers of the NLRP3 activation process are saturated fatty acids and ceramide species (52, 98, 101), whose circulating levels are reduced in obese animals and subjects in response to exercise. Interestingly, our above mentioned study (unpublished) revealed that the plasma levels of ceramides in mice were increased by feeding a HFD, an effect that was significantly attenuated by both endurance and resistance exercise. Exercise might also inhibit the priming step of NLRP3 activation in obesity through its ability to reduce ER stress (9), because ER stress is known to induce the priming step of NLRP3 activation via NF-κB activation (44). Considering that ROS, which are produced in response to ER stress, act as second hit signals leading to the assembly of the NLRP3 components into the active NLRP3 inflammasome and subsequent conversion of cytokine precursors into the active cytokines, exercise might also inhibit the NLRP3 inflammasome pathway in the activation step.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Convincing evidence exists that activation of the innate immune system in the course of obesity is mediated by metabolic signals, such as FFAs, through activation of PPRs like TLR4 thereby leading to stimulation of critical inflammatory signaling cascades that interfere with insulin signaling. The present review provides evidence from the literature showing that exercise is a successful strategy to inhibit obesity- and FFA-induced activation of inflammatory signaling pathways being responsible for attenuating obesity-induced metaflammation **(Fig. 1)**. One important mechanism of exercise on inhibition of obesity-induced activation of inflammatory signaling pathways is suppression of FFA-induced expression and activation of TLR4, a receptor that is critically involved in the activation of the innate immune system by FFAs in obesity. As a consequence of this, it was consistently found in several studies that both acute and chronic exercise and different forms of exercise (running, swimming) inhibit the IKKβ/NF-κB pathway and improve insulin signaling in metabolic tissues of obese animals and humans. In contrast, only two studies are available in the literature investigating the potential of exercise training to attenuate obesity-induced ER stress, with however opposing outcomes; whereas one study reported an inhibition of obesity-induced ER stress by swimming training, the other study revealed an exacerbation of obesity-induced ER stress by running training. Thus, future studies are necessary to clarify the different mechanisms of swimming and running exercise in the regulation of ER stress in obesity. With regard to the NLRP3 inflammasome pathway several human studies reported a decrease in the plasma levels of IL-18 in obese subjects in response to chronic exercise representing at least indirect evidence for inhibition of obesity-induced NLRP3 inflammasome pathway. However, regarding that no published studies are available exploring the direct effect of exercise on obesity-induced activation of the NLRP3 inflammasome pathway future studies are required to close this gap of knowledge. Thus, despite evidence from the majority of published studies that exercise has counteractive effects on obesity-induced activation of inflammatory signaling pathways further studies are necessary to establish the most successful exercise protocol (type, intensity, duration) for preventing and treating obesity and its co-morbidities.

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