MedCrave
Step into the World of Research
Journal of Nutritional Health & Food Engineering

Review Article
Open Access
CrossMark

The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females

Abstract

Exercise-induced muscle damage (EIMD) occurs following unaccustomed exercise, usually involving eccentric muscle contractions. Eccentric exercise contractions may cause harsh morphological changes in the individual muscle fiber. Various interventions have been proposed to attenuate EIMD and DOMS. An intriguing proposed intervention involves 17-β estradiol (estrogen) as an anti-oxidant. Estrogen has a cyto-protective effect on the sarcolemma, which protects the muscle from oxidative-induced muscle damage known to occur with strenuous exercise. It has been theorized that estrogen has the functional capacity to act as a membrane stabilizer, Due to the supposed cytoprotective effects of 17-β estradiol (aka. estrogen), females are thought to be less predisposed to exercise-induced muscle damage than males. However, females may be more prone to muscle damage during the low estrogen point in their 28-35day cycle (follicular phase) compared to their high estrogen point (luteal phase). Numerous treatments have been proposed to minimize muscle damage and alleviate the symptoms of DOMS, but a clear beneficial treatment has not yet been identified. Another intriguing idea is that the anti-inflammatory and anti-oxidative properties of omega-3 (n-3) fatty acids may help counteract the inflammatory state associated with muscle damage and DOMS. The omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are of interest as they are common components of fish oil supplements and have been shown to be beneficial in improving some inflammatory conditions. Therefore, fish oil supplementation has been suggested to be important for cytoprotection due to its anti-oxidant potential for significantly decreasing markers of muscle damage. Therefore, fish oil supplementation may reduce oxidative stress, thereby augmenting cytoprotection throughout the course of the 28day menstrual cycle.

Keywords: eccentric exercise, muscle damage, estradiol, female, omega-3 fatty acids

Volume 2 Issue 6 - 2015

Sarah McKinley-Barnard, Darryn S Willoughby
Department of Health, Baylor University, USA

Correspondence: Darryn Willoughby, Ph.D, Baylor University, Department of Health, Human Performance, and Recreation, Waco, TX 76798, Tel 2547103504,
Email darryn_willoughby@baylor.edu

Received: September 20, 2015 | **Published:** October 12, 2015

Abbreviations: EIMD, exercise-induced muscle damage; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DOMS, delayed onset muscle soreness; HMB, β-hydroxyl-β-methylbutyrate; TNF, tumor necrosis factor; ROS, reactive oxygen species; EC, excitation-contraction; MAPK, mitogen-activated protein kinase; H2O2, hydrogen peroxide; NO, nitric oxide; FOXO, forkhead box; (Murf1, murine ring-finger-1; NIK, NF-κb inducing kinase; IKK, iκb kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; PFA, polyunsaturated fatty acids; LA, linoleic acid; COX, cyclooxygenase; LOX, lipoxygenase; PG, prostaglandins; TX, thromboxanes; LT, leukotrienes; HETE, hydroxyeicosatetraenoic acids; PPAR, peroxisome proliferator-activated receptor; CRP, c-reactive protein; MDA, malondialdehyde; TBARS, thiobarbituric acid-reactive substance

Introduction

It is well known that exercise-induced muscle damage (EIMD) occurs following unaccustomed exercise, usually involving eccentric muscle contractions. An eccentric muscle contraction occurs when an external force is greater than that being exerted by the working muscle, which results in lengthening of the muscle fiber.[1] During an eccentric muscle contraction, the muscle lengthens while it is trying to contract. There is a greater mechanical strain per muscle fiber with eccentric muscle contractions compared to concentric muscle contractions because fewer muscle fibers are recruited during these contractions.[2] During concentric muscle contractions, the muscle is doing the work, but during eccentric muscle contractions, work is done on the muscle by the external forces.[3] The ability of the muscle to resist force is approximately 30% higher during a maximal voluntary contraction compared to the muscle's ability to exert force during a concentric contraction.[4] However, while the muscle's ability to resist force is considerably greater during eccentric contractions, the metabolic cost and neural activation is smaller compared to concentric contractions.[2,5]

Eccentric exercise contractions may cause harsh morphological changes in the individual muscle fiber.[6] During concentric muscle contractions, the myosin cross-bridges make repeated, constant connections with actin filaments for the duration of the muscle contraction. However, eccentric muscle contractions cause the actin filaments to be pulled in opposite directions by the external forces acting on the working muscle fibers as opposed to the center of the myosin filament as with concentric muscle contractions.[6]

Numerous studies have determined the symptoms of EIMD, some of which include soreness,[3,7] decrease in range of motion of

affected limb,[3,8] decrease in muscular strength,[3,9] leakage of myofiber proteins into the blood, particularly creatine kinase,[3,10,11] and structural damage and inflammation.[3] The most frequently reported symptom of EIMD is delayed onset muscle soreness (DOMS).[12] Usually, DOMS is associated with a feeling of discomfort that accompanies EIMD. Within the first 24hours following exercise, the intensity of DOMS increases, then peaks between 24 and 72hours, and finally subsides completely between 5 and 7days following cessation of exercise.[13] For elite athletes, EIMD and DOMS often occur due to a sudden increase in volume or intensity of their workout, while for sedentary individuals, a single bout of eccentric exercise may result in EIMD and DOMS.[14]

While it has been shown that eccentric exercise is potentially damaging, it has also been shown to be beneficial to the overall gains of the muscle. Eccentric exercise has been shown to produce greater hypertrophy gains than concentric contractions,[15–19] increase eccentric contraction-specific strength[16,20] (Figure 1), and eccentric contractions occur at a lower metabolic cost, resulting in a smaller stress on the cardiovascular system compared to concentric contractions.[21,22] These benefits of eccentric exercise have allowed for numerous studies to advocate the importance of including eccentric exercise in training to maximize gains in hypertrophy and strength. However, the damaging effects of eccentric exercise, which are thought to be necessary for adaptive muscle remodelling,[23,24] can affect later exercise sessions due to the symptoms EIMD can cause. As a result, recent research has begun looking into interventions that may alleviate negative symptoms of EIMD, particularly DOMS.

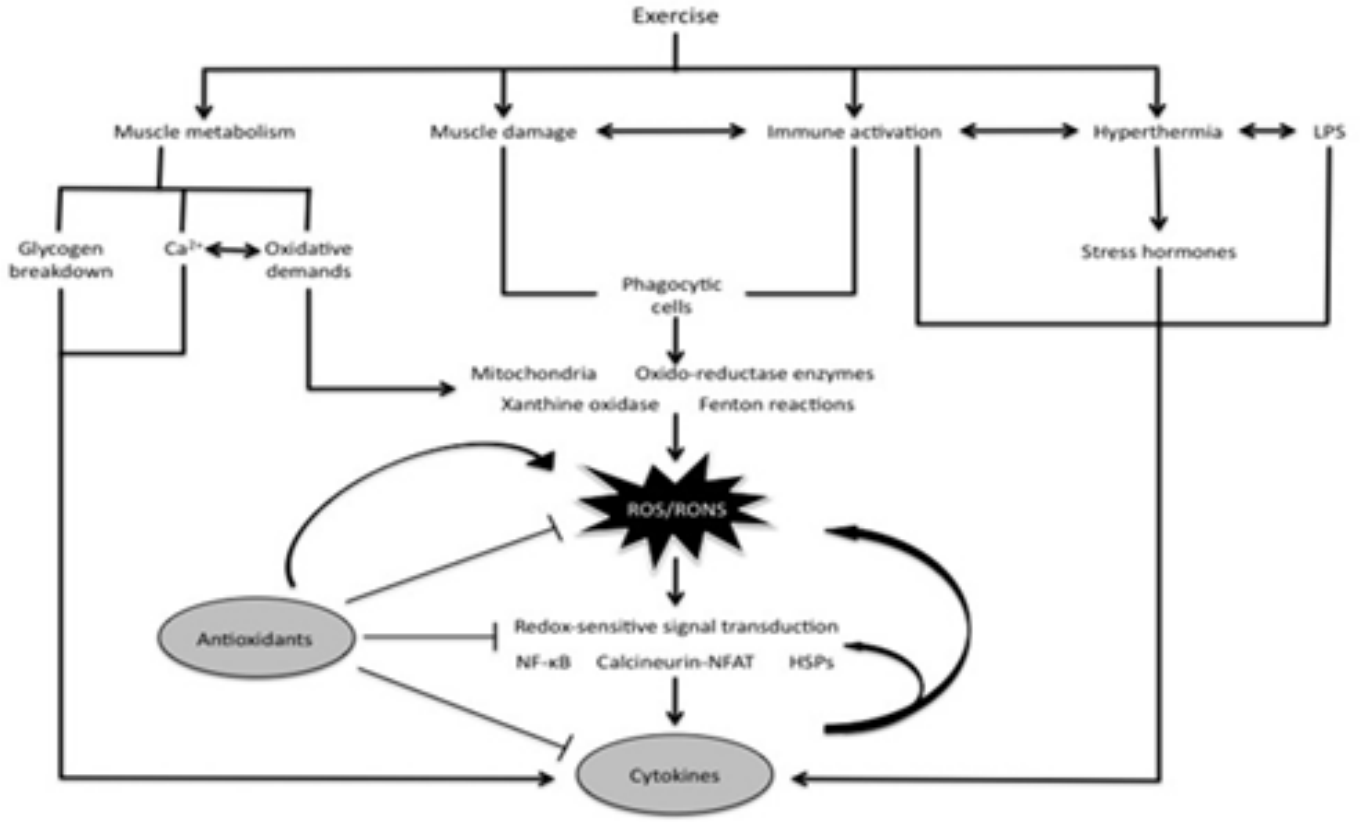

Figure 1 Interactions between exercise, ROS/RONS, antioxidants, and cytokines.

Exercise causes mechanical and metabolic stresses on skeletal muscle, thereby inducing an oxidative and inflammatory response. As a result, ROS accumulation occurs and activates NF-κB, in addition to calcineurin-nuclear T factor of activated T cells (NFAT) signaling and heat shock proteins (HSPs). Modified from Peake et al.,[20]

Various interventions have been proposed to attenuate EIMD and DOMS, including cryotherapy, stretching, anti-inflammatory drugs, massage, exercise, β-Hydroxyl-β-Methylbutyrate (HMB), carbohydrates and proteins, and anti-oxidants. The most intriguing of these proposed interventions involves 17-β estradiol (estrogen) as an anti-oxidant. Estrogen has a cyto-protective effect on the sarcolemma, which protects the muscle from oxidative-induced muscle damage known to occur with strenuous exercise. It has been theorized that estrogen has the functional capacity to act as a membrane stabilizer, which attenuates creatine kinase and myoglobin release from muscle cells following exercise.[25]

Numerous treatments have been proposed to minimize muscle damage and alleviate the symptoms of DOMS, but a clear beneficial treatment has not yet been identified. Another intriguing idea is that the anti-inflammatory and anti-oxidative properties of omega-3 (n-3) fatty acids may help counteract the inflammatory state associated with muscle damage and DOMS.[26] The omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are of interest as they are common components of fish oil supplements and have been shown to be beneficial in improving some inflammatory conditions.[27]

Citation: McKinley-Barnard S, Willoughby DS. The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females. *J Nutr Health Food Eng.* 2015;2(6):205–219. DOI: 10.15406/jnhfe.2015.02.00082

Circulating tumor necrosis factor-alpha (TNF-α) is a marker of acute and systemic stress-induced inflammation and has been previously shown to be released in response to eccentric exercise.[28–30] Upon its release, TNF-α binds to its trans-membrane receptor, thereby up-regulating the nuclear factor-kappa b (NF-κB) signaling cascade. The signaling pathway is responsible for up-regulating proteolysis and apoptosis within skeletal muscle.[31] Therefore, being able to attenuate this cascade with fish oil supplementation could play a beneficial role in minimizing muscle proteolytic activity that has been previously shown to accompany exercise-induced muscle damage.[32,33]

To date, there is a paucity of studies that have examined the relationship between fish oil supplementation, DOMS, and the associated inflammatory response. Furthermore, in regards to the potential cyto-protective effects in females, no published data appear to exist. The studies that do exist typically employ a muscle-damaging workout followed by subjective measurements of pain and inflammatory responses during the following days while using older, untrained individuals. The results of these studies have been mixed. Some of the studies have shown fish oil supplementation to be effective at relieving the perceived pain associated with DOMS after exercise,[34,35] while others have shown no effect of fish oil on inflammatory markers and DOMS.[36–38] Additionally, the extent of muscle damage contains individual variability and may be potentially explained by factors including the exercise type and intensity, fitness level, age, and gender.[25] However, the limited number of studies and mixed results warrants further investigation into the possible benefits of fish oil on markers of muscle damage in females, particularly during periods of low and high estradiol levels.

Mechanisms of exercise-induced muscle damage

The exact mechanisms of EIMD are not exactly known, but Armstrong[39] has proposed four stages of muscle injury that are still widely accepted: initial events, autogenic processes, phagocytic processes, and regenerative phase.

Initial phase

The initial events of muscle damage are thought to occur either by mechanical or metabolic stressors,[39] and may occur simultaneously.[12] Increased tension, or an imbalance in tension that is associated with eccentric contractions may disrupt the sarcolemma, the sarcoplasmic reticulum (both of which cause a disruption in skeletal muscle calcium homeostasis), and myofibrillar structures.[39] During repeated eccentric contractions it is proposed that the number of disrupted sarcomeres increases until membrane damage occurs (Figure 2).[40] Once membrane damage occurs, damage to the elements of excitation-contraction (E-C) coupling becomes noticeable.

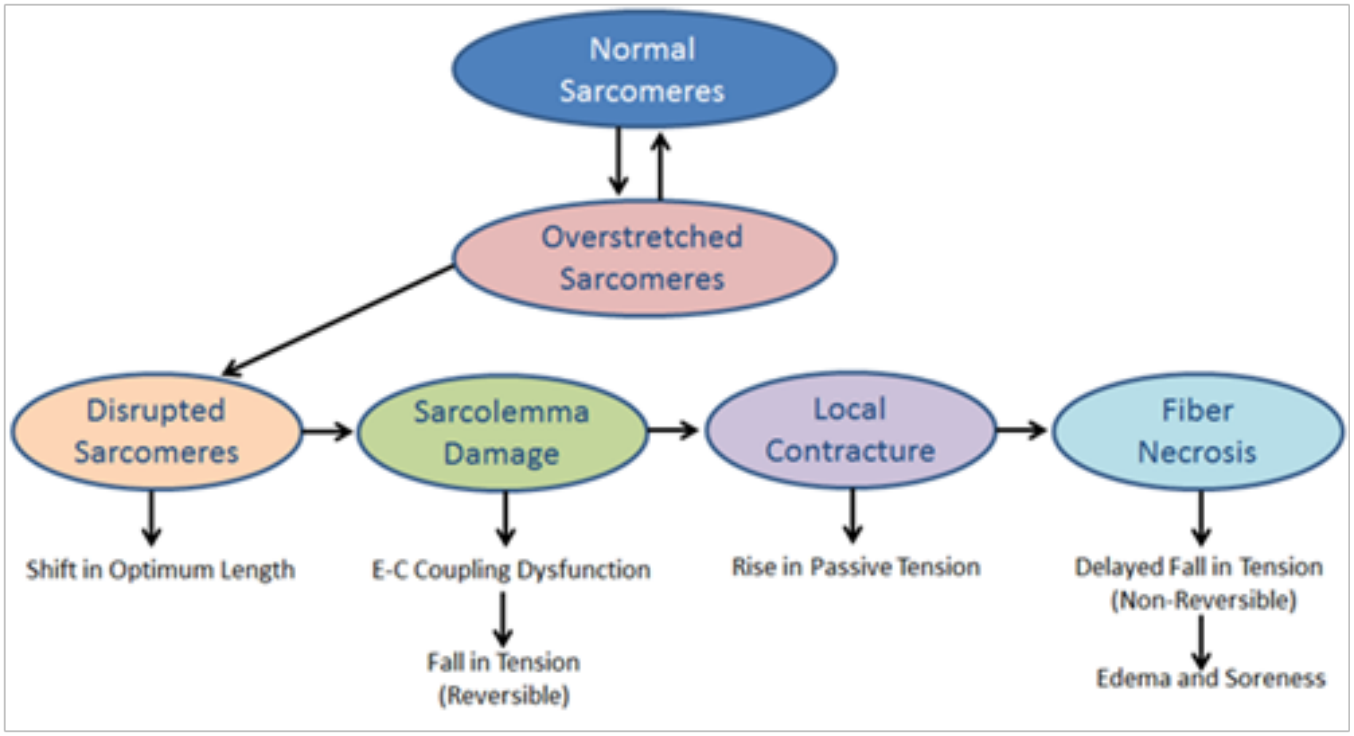

Figure 2 Postulated series of events leading to muscle damage from eccentric exercise.

During an active lengthening, longer, weaker sarcomeres are stretched onto the descending limb of their length-tension relation where they lengthen rapidly, uncontrollably, until they are beyond myofilament overlap and tension in passive structures has halted further lengthening. Repeated overextension of sarcomeres leads to their disruption. Muscle fibers with disrupted sarcomeres in series with still-functioning sarcomeres show a shift in optimum length for tension in the direction of longer muscle lengths. When the region of disruption is large enough it leads to membrane damage. This could be envisaged as a two-stage process, beginning with tearing of t-tubules. Any fall in tension at this point would be reversible. However, it would be followed by damage to the sarcoplasmic reticulum, uncontrolled $Ca2^{+}$ release from its stores and triggering of a local injury contracture. That, in turn, would raise muscle passive tension. If the damage was extensive enough, parts of the fiber, or the whole fiber, would die. This fall in tension would not be recoverable. Breakdown products of dying and necrotic cells would lead to a local inflammatory response associated with tissue edema and soreness. Modified from Proske and Morgan.[40]

Citation: McKinley-Barnard S, Willoughby DS. The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females. *J Nutr Health Food Eng.* 2015;2(6):205–219. DOI: 10.15406/jnhfe.2015.02.00082

Metabolic stress on the muscle includes increased temperature, increased acidity, insufficient mitochondrial respiration, and oxygen free radical/reactive oxygen species (ROS) production.[39] ROS production has been proposed in a number of paradigms to be related to muscle inflammation and injury specifically.[41] Tissue that is highly metabolically active produces ROS, which can cause irreversible damage to the cell. Disruption in calcium homeostasis can occur when the sulfhydryl groups of the ATPase pump is oxidized by free radicals, causing a reduction in the rate of calcium uptake by the sarcoplasmic reticulum. The increase in acidity that accompanies ROS production during strenuous activity affects the ability of the sarcoplasmic reticulum to take up calcium. This is due to the free hydrogen ions and the calcium ions competing for the binding sites of calcium on the ATPase pump.[42]

Disruption of calcium homeostasis: Disruption of calcium homeostasis is a result of the events of the initial phase of EIMD, and it occurs whether the stressor on the muscle is mechanical or metabolic. The disruption in calcium homeostasis results in a subsequent rapid activation of autogenic destructive events in the muscle fiber. It is believed that the loss of calcium from the cell plays a major role in muscle injury and subsequent repair processes.[39]

Calcium is necessary for normal muscle fiber function, but increased levels of calcium can be detrimental to the muscle fiber, resulting in cell dysfunction or death. The rapid increase in intracellular calcium levels is thought to be an important step in the cascade of events that result in muscle fiber damage following eccentric exercise. Increased levels of calcium in the muscle fiber results in ultrastructural changes in the muscle fiber, including inflamed and disrupted mitochondria, dilated t-tubules and sarcoplasmic reticulum, and disruption of the myofilaments.[42] Data from McArdle & Jackson[43] has demonstrated that an increase of calcium in the muscle fiber causes damage to the myofilaments, which is congruent with data from Byrd[42] and Amelink et al.,[44] whom both showed that an inhibition of calcium influx across the sarcoplasmic reticulum following exercise decreases damage in the muscle fiber. The elevated levels of calcium in the muscle fiber cause a release of enzymes through activation of phospholipase A2. This may induce injury to the sarcolemma through production of leukotrienes and prostaglandins via ROS production.[39] As a result, this affects the fluidity of the membrane, and causes an efflux of intracellular enzymes and lysosomal enzymes to flow freely across the membrane.[12] Increased calcium levels have also been shown to disrupt the E-C coupling process, which may be related to the decrease in force production associated with eccentric exercise.[45]

Autogenic processes

Following some event that initially causes some structural component of the muscle fiber to fail, meaning a physical force causes a disruption of the normal permeability barrier to calcium outside of the cell, calcium is able to enter the muscle fiber at the site of damage, and potentially overwhelm the buffering systems for calcium in the fiber. Once calcium levels in the cytosol are increased to a certain level, are elevated for a sufficient period of time, or increased within specific compartments of the muscle fiber, various degradative mechanisms are activated in the muscle fiber.[1]

Various processes have been postulated to explain how skeletal muscle may be damaged due to an elevation of calcium in the muscle fiber. These processes include stimulation of calcium-activated proteases, activation of lysosomal proteases, overload of mitochondria, and activation of lipolytic enzymes.[12] One of the most important processes seems to be the activation of calcium-dependent proteases, specifically calpain.[43] It is believed that increased levels of calcium in the muscle fiber stimulate proteases, specifically calpains, which act directly on the proteins in the membranes and specifically on the Z-discs.[42,46]

There are two types of calpains, type 1 and type 2, which are activated depending on the concentration of calcium in the cytosol. The type 1 isoform of calpain is activated when there are micromolar quantities of calcium, while the type 2 isoform is activated when there are millimolar amounts.[47] These proteases are not specific to any protein or peptide sequence, but are associated with the degradation of particular structures in the muscle cell.[48] The isoenzymes of calpain are usually localized in the I and Z bands of the muscle fiber.[49] Calpain degrades Z-discs by digesting the proteins zeelin 1 and 2, which anchor α-actinin in the Z-disc.[50] Calpain cleaves a variety of protein substrates including cytoskeletal and myofibular proteins, making calpain-stimulated degradation thought to contribute to the changes in structure and function of the muscle that is a result of eccentric exercise. Activation of calpain results in selective proteolysis of various contractile, structural, and metabolic elements.[49]

Proteins in the myofibril may also be degraded by lysosomal proteases. Increases in calcium concentration in the cytosol activate calmodulin, which is associated with lysosomal vesicles[51] and phospholipase A2, which increases production of prostaglandin E2 and thereby stimulates activity of lysosomal proteases.[52] Phospholipase A2 is located in the sarcolemma, membranes of organelles, cytosolic compartment, and in lysosomes.[53] It is the first enzyme in the pathway that utilizes membrane phospholipids as substrate for the production of arachidonic acid, which subsequently produces prostaglandins, leukotrienes, and thromboxanes.[1]

Phagocytic processes

Damage to skeletal muscle initiates at what is termed the acute phase response,[54] which facilitates anti-bacterial and anti-viral responses prior to promoting the clearance of debris.[12] Inflammation is characterized by the movement of fluid, leukocytes, and plasma proteins in response to damage into the tissue (in this case skeletal muscle).[55] Inflammatory cells migrate to the site of injury in the muscle to remove cellular debris, and myogenic cells migrate to the site of injury to initiate mechanisms to repair the damaged muscle.[56] These cells penetrate the muscle by means of specific cytokines, which are small polypeptides that are considered an important link between neuroendocrinal and immunological systems that are involved in inflammation and acute phase response, among others.[14,55] Specific cytokines such as interleukin (IL)-1, interferon, interleukin-2, interleukin-6, and tumour necrosis factor-α (TNF-α) are believed to be the primary mediators of inflammation.[57] IL-1 is thought to influence muscle inflammation, play a role in stimulating protease synthesis and induce the expression of other cytokines such as IL-2, IL-3, IL-6, and TNF-α. TNF-α and IL-1 have been shown to have mechanisms that coincide with each other, which ultimately can lead to leucocyte adhesion and stimulating leucocyte activity.[56]

Leukocytes are believed to have three main functions within the damaged muscle and repair cycle: neutrophils and macrophages attack and breakdown debris; macrophages removal cellular debris; and macrophages regenerate cells.[46,56] Leukocytes are drawn to injured skeletal muscle fibers through several chemotactic factors including leukocytes that are already at the site of injury, calpain fragments, and/or cytokines at the site of injury. Neutrophils are usually the first leukocytes to arrive at the site of injury. These cells then release a signal to intensify the response of other neutrophil cells

and mononuclear cells.[12] Neutrophils produce superoxide and ROS products via a respiratory rupture that is catalyzed by NADPH oxidase in the plasma membrane. Pyne[14] determined that neutrophils do not have the ability to distinguish healthy tissue from foreign antigens, so it destroys healthy aspects of the cell as well as the damaged cell debris. While the phagocytic processes mainly include further disruption and destruction of the muscle fiber, they are necessary precursors to the regeneration phase of growth and restoration of the damaged tissue and repair of normal cell function.

Regenerative phase

During the phagocytic phase, there is a division of surviving satellite cells. These satellite cells mature into myoblasts, and fuse to form new myotubes.[12] However, it is not clear how the satellite cells are stimulated to divide. Numerous studies have postulated that macrophage infiltration seems to be a critical prerequisite for regeneration, especially for proliferation of satellite cells.[58–60]

Oxidative stress and redox-signaling pathways

Oxidative stress is a factor that is apparent in exercise-induced muscle damage. Oxidative stress is defined as the imbalance between production of ROS and the ability to detoxify reactive intermediates or to repair subsequent damage by an adequate anti-oxidant defense.[61] This can lead to direct damage of cellular mechanisms by oxidation including lipids, proteins, and DNA. Furthermore, oxidative stress can act as a regulator of the acute phase inflammatory response.[31] After injury, infiltrating leukocytes exert antiseptic protection of muscle by releasing ROS through oxidative burst activation of NADPH oxidase (Figure 3).[62] This process results in marked perturbations of redox status in muscle fibers.[63] Consequently, pro-inflammatory cytokines, including TNF-α, are released by neutrophils and injured muscle fibers, thereby activating ROS-generating enzymes such as xanthine oxidase.[64] When ROS production is greater than the capacity of the antioxidant defense system, an oxidative stress occurs resulting in many cellular contents suffering oxidation due to ROS attack[65] and subsequent activation of nuclear factor kappa B (NF-κB), activator protein 1 (AP-1), and forkhead box (FOXO) proteins. Additionally, increased levels of ROS results in oxidation of several proteins in the excitation-contraction mechanism,[66] and the production of protein reactive carbonyl derivatives lead to a loss of catalytic activity and increased vulnerability to protein breakdown.[67]

ROS has been shown to influence the activity of transcription factor binding through various ways including activating kinases to stimulate a signaling cascade through sequential enzyme phosphorylation, regulating anti-oxidant signaling by modulating phosphatase activity, and regulating the synthesis and degradation of transcription factors.[68] Allen & Tresini[69] determined that almost half of the effects of ROS reported involve members of the mitogen-activated protein kinase (MAPK) and NF-κB pathways. They also concluded that hydrogen peroxide (H_2O_2), nitric oxide (NO), calcium (Ca2+), and cytokines are among the agents that are capable of assisting as signaling molecules in response to oxidative stress. These messengers transfer signals from the surface of the cell to the nucleus in the cell to stimulate gene expression.[68] H_2O_2 has been indicated as the most common messenger in response to oxidative stress, contributing to more than 50% of occurrences from research studies.[69]

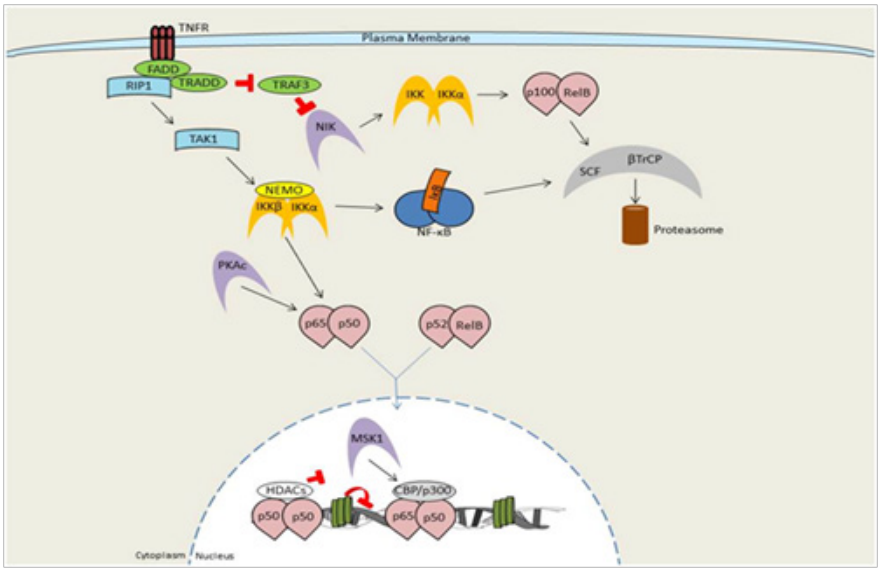

Figure 3 Overview of NF-κB signaling by TNF receptor signaling.

The classical, canonical, signaling pathway is initiated by the binding of either TNF-α, IL1, or LPS to its receptor and the subsequent sequential recruitment of the adaptors TRADD, RIP, and TRAF to the sarcolemma. IKK complex assembly and recruitment to the sarcolemma occurs between IKKα, IKKβ, and IKKγ, thereby resulting in IKKβ phosphorylation and activation. IKKβ then phosphorylates IκBα to promote polyubiquination and subsequent immediate proteasomal degradation through β-TrCP. Signaling by members of the TNF receptor superfamily, such as TNFR1, leads to recruitment of the adaptor proteins FADD and TRADD, TRAF family members (TRAF2, 5, and 6), and the kinase RIP1. The TRAF/RIP complex recruits and activates TAK1, which induces activation of the IKK complex and subsequent downstream NF-κB signaling. Upon stimulation, a subset of TNFR superfamily members that bind to TRAF3 (CD40, LTβR, BAFFR) induce TRAF3 degradation resulting in accumulation of the kinase NIK. NIK then undergoes constitutive degradation in the absence of stimulation. Accumulated NIK phosphorylates and activates IKKα. IKKα, thereby inducing processing of the NF-κB family member p100 into p52. At this point p52-containing NF-κB complexes become activated (prototypically RelB:p52). Modified from Hayden et al.,[62]

Citation: McKinley-Barnard S, Willoughby DS. The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females. *J Nutr Health Food Eng.* 2015;2(6):205–219. DOI: 10.15406/jnhfe.2015.02.00082

Cellular responses that are stimulated or influenced by ROS have been proposed to be classified into several categories that include modulation of cytokines, hormone release and action, growth, ion transport, transcription, and apoptosis.[70] Conversely, Jackson et al.,[71] proposed that cellular responses that are influenced by ROS was directly related to changes in cell proliferation, immune function, stimulation of apoptosis, or a combination of these. It can be concluded that it is still unclear whether ROS does in fact influence all, if any, of these cellular responses in skeletal muscle. Powers & Jackson[72] suggest this remains unclear because the predominant area of ROS research has focused mainly on the contractile properties of skeletal muscle.

As stated previously, the NF-κB and MAPK signaling pathways are considered the most important for the cells to cope with oxidative stress. Particularly, NF-κB has been shown to be a redox-regulated factor that is most affected by ROS.[73] Hansen et al.,[74] determined that activation of ROS appears to involve oxidation of cysteine residues in upstream activators of NF-κB. They also showed that this is usually prevented by anti-oxidants or other reducing agents.

NF-κB

NF-κB is a ubiquitously expressed transcription factor that is considered vital to numerous cellular processes.[75,76] It has been suggested that NF-κB directly modifies hundreds of gene products, including genes that encode cytokines, chemokines, cell adhesion molecules, growth factors, immunoregulatory receptors, acute-phase and stress response proteins, cell surface receptors, transcription factors, and several enzymes involved in protein degradation by the ubiquitin-proteasome system, as well as regulators of redox status, apoptosis, disuse atrophy, and host defense.[77,78] The NF-κB family is comprised of five genes that code for protein subunits, RelA/p65, RelB, c-Rel, p50, and p52.[75,76,79,80] In humans, the proto-oncogene c-Rel is a protein that is encoded by the REL gene (v-rel avian reticuloendotheliosis viral oncogene homolog). The c-Rel protein is a member of the NF-κB family of transcription factors and contains a Rel homology domain (RHD) at its N-terminus and two C-terminal trans-activation domains. The inhibitor family of NF-κB (IκB) consists of seven proteins including IκBα, IκBβ, IκBε, IκBγ, B-cell lymphoma 3-encoded protein (BCL-3), and precursor proteins p100 and p105.[76,79–81] The most commonly described forms of NF-κB are the p50/p65 heterodimer and p50/p50 homodimer complexes, followed by p50/c-Rel and p52/RelB.[75,82] Unlike the other genes, p50 and p52 genes lack transcriptional activation domains, which generates p50 and p52 homodimers that function as gene repressors by blocking DNA consensus sites.[75–77,83]

Prior to activation, NF-κB dimers are held in the sarcoplasm bound to inhibitory proteins, termed inhibitor of NF-κB (IκB).[75,77,80] While NF-κB is bound to IκB, nuclear translocation is averted, which maintains the inactive state of NF-κB in the cytoplasm.[77] However, when IκB proteins are degraded following stimulation of the cell, nuclear entry of NF-κB dimers becomes favoured.[76] Specifically, once the cell is stimulated, the IκBα protein is quickly phosphorylated at serine 32 and 36, which activates poly-ubiquination and degraded by the 26S proteasome, thereby allowing nuclear entry of NF-κB dimers.[80] NF-κB dimers are then free to travel to the nucleus where they bind to κB sites within target gene promoters. NF-κB, along with other transcription factors, then regulates transcription of genes.[81]

Activation of the NF-κB complex occurs in response to various stimuli, including infection, exposure to pro-inflammatory cytokines, mitogens, growth factors, biomechanical stressors, and oxidative stressors. There are several pathways by which NF-κB can be activated following ligation of different receptor families, including tumor-necrosis factor receptor (TNFR) and interleukin-1 receptor (IL-1R). NF-κB complex activation also includes the activation of several intermediate kinases, including NF-κB inducing kinase (NIK), which is a member of the MAPK pathways. These intermediate kinases act as distinct signaling proteins that all unite on the IκB kinase (IKK) complex.[76,77]

The IKK complex controls the breakdown of IκB proteins through regulation of the phosphorylation of serine 32 and 36 on IκBα, and serine 19 and 23 on IκBβ. This phosphorylation results in K48-linked polyubiquitination by the SCFβTrCPE3 ubiquitin ligase complex on lysine 21 and 22 of IκBα, which is an ATP-dependent occurrence that quickly targets the breakdown of these proteins by the 26S proteasome.[75,76]

It has been determined that murine C2C12 skeletal muscle cell lines contain p65/p50 in their nucleus, which is the most commonly studied form of NF-κB. In the myoblast cell line C2C12, NF-κB binds on κB sites of the cyclin D1 promoter. This then allows for transcription regulation, leading into the S phase of the cell cycle. During myogenesis, binding activity of NF-κB on cyclin D1 is decreased. This suggests that NF-κB is a critical element regulating transition to the differentiation stage from the proliferation phase. During atrophic conditions, NF-κB binds on the promoter of murine ring-finger-1 (MuRF1), which is an E3 ubiquitin ligase.[84] This increases expression of MuRF1, suggesting NF-κB regulates the ubiquitin-proteasome system (UPS), resulting in muscle wasting.[75,84]

Another potential mechanism by which increased NF-κB activity leads to skeletal muscle wasting is the possibility of NF-κB increasing the expression of inflammation-related molecules, which either directly or indirectly enhances muscle wasting. Pro-inflammatory cytokines, including TNF-α, THF-like weak inducer of apoptosis (TWEAK), IL-1, and IL-6 are major inducers of muscle wasting. Evidence has shown that NF-κB regulates expression of cytokines, chemokines, and cell-adhesion molecules.[85] It has also been determined that NF-κB inhibition promotes regeneration of skeletal muscle by limiting the inflammatory response.[86]

Various physiological stressors such as disease and exercise, which causes muscle injury, are known to induce oxidative stress. After injury, infiltrating leukocytes exert antiseptic protection of muscle by releasing reactive oxygen species (ROS) through oxidative burst activation of NADPH oxidase.[62] This process results in marked perturbations of redox status in muscle fibers.[63] Consequently, pro-inflammatory cytokines, including TNF-α, are released by neutrophils and injured muscle fibers, thereby activating ROS-generating enzymes such as xanthine oxidase.[64] When ROS production is greater than the capacity of the antioxidant defense system, an oxidative stress occurs resulting in many cellular contents suffering oxidation due to ROS attack[65] and subsequent activation of NF-κB, activator protein 1 (AP-1), and forkhead box (FOXO) proteins. Additionally, increased levels of ROS results in oxidation of several proteins in the excitation-contraction mechanism,[66] and the production of protein reactive carbonyl derivatives lead to a loss of catalytic activity and increased vulnerability to protein breakdown.[67]

It is well known that cellular stress is an activator of the NF-κB classical pathway, specifically ROS formation. This has been shown in various research studies through a variety of ways that include exposure of certain cell types, such as L6 myocytes, to H2O2 leading to the activation of the NF-κB pathway; activators of NF-κB, such as TNF-α, IL-1, and LPS can all lead to an increase in H2O2 levels in the cell; and treatment with anti-oxidants, such as glutathione (GSH), prevents NF-κB activation.[68] Sen et al.,[87] were the first to demonstrate that TNF-α-induced activation of NF-κB in L6 myocytes, which was augmented by conditions of oxidative stress. It was also determined that inhibition of NF-κB activity by the antioxidant pyrrolydinedithiocarbamate, coupled with an increase in intercellular adhesion molecule -1 (ICAM-1) expression, suggested that the intracellular redox status was critical in activation of NF-κB, nuclear translocation, and subsequent gene transcription regulation of proteolytic genes.[88] Li et al.,[79] also found that TNF-α led to ROS-mediated NF-κB activation, resulting in a decrease of total protein in skeletal muscle with a specific loss of myosin heavy chain. The enhanced muscle protein degradation was coupled with TNF-α-dependent stimulation of total ubiquitin conjugation of myotube proteins.[79]

The NF-κB signaling pathway has been the most widely studied pathways involving regulation of the redox-sensitive pathways.[89] However, the NF-κB signaling pathway works in conjunction with the MAPK signaling pathway and its proteins ERK, JNK, and p38 specifically, which will be briefly noted.

MAPK

The main function of the MAPK pathway is to facilitate growth, metabolism, transcription, differentiation, translation, and remodeling. MAPK is known to have a complicated hierarchy that is intertwined with other kinases, such as ERK and JNK, which are up-regulated by their own respective kinases.[69] The main activators of the MAPK pathway include growth factors, inflammatory cytokines, and phorbol esters. In the ERK and JNK pathways, Ras has a critical role in the activation of MAPK. Ras stimulates the translocation of Raf-1, which controls MAPK kinase (MEK/MKK).[68] TNF-α and IL-1 are able to bypass the Ras pathway through increasing the level of H2O2 in the cytosol, which activates several forms of protein kinase C (PKC).[90] It has been suggested that PKC is a pivotal enzyme activating MAPK pathways through stimulation of MEK/MKKs, as well as the NF-κB pathway through NIK activation.[68] This shows the importance of crosstalk between the NF-κB pathway and MAPK pathways, since both pathways may directly or indirectly affect each other.

MAPK can be activated by cytokines and stress, which stimulates inflammation, degradation by phospholipase A2, and apoptosis.[69] In this regard, MAPK regulates gene expression mainly under the control of NF-κB signaling, such as through anti-oxidant enzymes. It is also worth noting that adaptations in skeletal muscle such as mitogenesis, hypertrophy, and transformation of fibers are regulated by MAPK signaling, and have been shown to play a critical role in determining homeostasis of cellular oxidant-anti-oxidant mechanisms.[68]

Estrogen and muscle damage

Recently, the potential protective role of estrogen from muscle damage has been of growing interest. However, it is still unclear as to whether estrogen really does play a protective role of muscle damage in humans. Numerous animal studies have supported the suggestion that estrogen has the potential to alleviate indicators of EIMD and inflammation. Conversely, human research has yielded more conflicting results. The discrepancies in human research of estrogen and muscle damage has mainly been attributed to differences in age, fitness levels, exercise protocols, and focus on sex-based differences as opposed to estrogen-specific effects.[25] Further research is needed in this growing area of interest.

An overview of estrogens

Estrogens describe a group of 18-carbon steroids molecules that are secreted mainly by the ovaries in females, and the testes in males to a much lesser extent.[91] While estrogens are mainly involved in the development and maintenance of normal reproductive and sexual function, they also have other biological effects in various physiological systems such as the cardiovascular system, musculoskeletal system, immune system, and central nervous system.[92] Estrogen refers to three steroid hormones that are similar in structure that include estradiol-17β (E2), estrone (E1), and estriol (E3).[12] Of these three steroid hormones, E2 is the main estrogen in humans, as well as the one with the most estrogen-like properties.[93] The other two estrogens, E1 and E3, have been shown to be more tissue-specific and are found in much smaller quantities than E2, making them less studied in humans.[94]

The protective role of estrogen has already been demonstrated in various physiological systems in humans. With respect to skeletal muscle, estrogen has been shown to exert protective effects, but the mechanisms by which this occurs remains unclear. Three hypotheses have been proposed to explain estrogen's influence on skeletal muscle: 1) estrogen is thought to have a high anti-oxidant capacity, and may have the ability to forage ROS and stimulate the expression of anti-oxidant enzymes, which limits oxidative damage;[95] 2) estrogen has a similar structural property to cholesterol, possibly allowing it the ability to intercalate within membrane phospholipids similar to cholesterol and exert a membrane-stabilizing effect;[93] and, 3) the discovery of three types of estrogen receptors (ERα, ERβ, and plasma membrane ER) has led to the determination that estrogen may have the ability to have gene regulatory effects.[96] However, less seems to be known about the latter two hypotheses.

Estrogen as an anti-oxidant

Estrogen is believed to have anti-oxidant characteristics due to the fact that its molecular structure is based on a carbon-ring structure, originating from a phenol species. Phenol species have at least one, or more, hydroxyl groups that give them the ability to reduce electrons.[97] Lipid peroxidation, which is a chain reaction that is mediated by ROS, can be stimulated by the hydroxyl radical attacking polyunsaturated fatty acids in membranes, resulting in oxidative damage[93] that affects the stability of the membrane. Numerous studies have shown that estrogen has anti-oxidant properties;[93,98–104] however, the mechanisms by which this occurs is not completely understood. It is believed that since estrogens possess a hydroxyl group on their phenolic ring in the same configuration and position as vitamin E, they may donate hydrogen atoms from the phenolic hydroxyl group, which would cease peroxidation chain reactions in a similar fashion to vitamin E.[105,106]

However, there is conflicting evidence as to whether estrogen has potential anti-oxidative protective effects. A study by Paroo et al.,[107] demonstrated anti-oxidative properties of estrogen following running exercise, while Feng et al.,[108] and Stupka & Tiidus[109] both demonstrated anti-oxidative properties of estrogen following muscle injury. Conversely, Tiidus et al.,[110] did not demonstrate anti-oxidative effects of estrogen in post-exercise indicators of oxidative stress. They

also showed that estrogen might reduce levels of other anti-oxidants, such as vitamin C and glutathione, in some muscle and tissues, arguing the point that estrogen may not exhibit anti-oxidative properties. Enns & Tiidus[25] suggest the inconsistencies of research in this area, particularly in humans, is likely due to the examination of chemical indicators of post-exercise oxidative stress in the blood as opposed to muscle biopsies, and focus on sex-based differences rather than the effects of estrogen. However, there is more evidence to support the argument that estrogen does exert anti-oxidative effects. Dernbach et al.,[111] showed that female rowers had lower levels of an oxidative stress marker in the blood after a 4-week training program compared to their male counterparts, while Ayres et al.,[112] demonstrated that amenorrhoeic females showed a significantly greater potential for lipid peroxidation following an acute bout of exercise compared to eumenorrhoeic females. Data has also been presented that suggest females may be protected more at certain points in their menstrual cycle. Kerksick et al.,[32] determined that females at the mid-luteal phase of their menstrual cycle had higher serum concentrations of superoxide dismutase (SOD), an anti-oxidant enzyme, compared to males following eccentric exercise. However, further research is needed in this area, as there are still questions and conflicting data.

Due to estrogen's potential anti-oxidant ability and configuration, it is thought to have membrane-stabilizing characteristics.[12] Wiseman & Quinn[113] have suggested that estrogen may decrease membrane fluidity and increase membrane stability to protect them from peroxidative damage in a similar fashion to cholesterol, which may be a mechanism of estrogen's anti-oxidant activity. Since steroid hormones are lipophilic, they can intercalate into the bilayer of the cell plasma membrane, which would potentially alter the fluidity and function of the membrane.[12]

As previously stated, pro-inflammatory cytokines increase following exercise, during muscle damage and the repair cycle. Yoshikawa & Yoshida[114] determined that vitamin E could inhibit NF-κB, which governs gene expression of various cytokines. They demonstrated that vitamin E prevents leukocyte-endothelial cell adhesion of inhibiting signaling transduction. Thus, they concluded vitamin E could have a protective effect against inflammation progression. Since estrogen has a similar structure and configuration to vitamin E, Kendall &Eston[12] suggest that estrogen could affect the expression of adhesion molecules, and potentially assuage any further damage by reducing neutrophil infiltration. However, in doing so, they also state this would inhibit the necessary inflammatory processes that leads to regeneration of the cell.

The effect of estrogen on creatine kinase

Creatine kinase is found in the cytosol and mitochondria of tissues where energy demands are high. There are two types of subunits of CK: muscle type (M) and brain type (B). These two subunits form three tissue-specific isoenzymes: cardiac muscle (CK-MB), skeletal muscle (CK-MM), and brain (CK-BB). CK forms the core of the phosphocreatine (PCr) system. In the PCr system, the cytosol isoenzymes are closely linked to glycolysis and produces ATP.[115] The mitochondrial CK (MtCK) is closely linked to the electron transport chain (ETC) and can use ATP in the mitochondria to regenerate PCr. This system is important for the production and maintenance of energy supply, and is involved in the metabolic feedback regulation of respiration.[116] Skeletal muscle CK can account for as much as 20% of the soluble sarcoplasmic protein in muscle.[115]

One of the most common markers of muscle damage, specifically muscle membrane disruption, is the appearance of CK in the blood.[117] A significant difference in CK activity has been shown between males and females at baseline and following muscle injury. In these studies, this difference has been attributed to estrogen effects. Amelink & Bär[118] showed that CK levels were significantly higher in male rats compared to female rats following muscle injury, which they attributed to the presence of estrogen. Further data from this group also showed a direct inverse relationship between estrogen supplementation and CK release in normal male and female rats, as well as ovariectomized female rats.[119] Data from this group suggest that the membrane-stabilizing effects of estrogen may assuage post-exercise CK release from skeletal muscle. They also showed that circulating CK levels could indirectly reflect changes in exercise-induced muscle membrane disruption. However, it is unknown whether the reduction in efflux of CK is an indication of increased membrane stability or if, in fact, the muscle is receiving less damage.

Estrogen and the inflammatory response

It has been suggested that since estrogen may be responsible for differences in gender-related vulnerability of muscle to EIMD, and may act as an anti-oxidant, the effects of estrogen administration on phagocytic infiltration into the muscle fiber following exercise should be examined.[120] Tiidus & Bambardier[120] hypothesized that a decrease in infiltration of neutrophil and macrophages may reduce the time-course and severity of the inflammatory response of the muscle following exercise, and potentially boost regeneration. They measured post-exercise tissue myeloperoxidase activity in male and female rats that were supplemented and not supplemented with estrogen. Their results showed that female rats had significantly attenuated infiltration of neutrophils into skeletal muscle 24-hours post-exercise when compared to male rats. However, when the male rats were supplemented with estrogen, they showed the same attenuation of neutrophil infiltration 24hours post-exercise as the female rats. These results suggest that estrogen significantly affects post-exercise infiltration of leukocytes into skeletal muscle. Numerous other data from these researchers have confirmed these results.[96,109,121,122] However, the mechanism(s) via which this occurs is still unclear.

St. Pierre Schneider et al.,[123] examined the time course and leukocyte concentration in injured soleus muscle in male and female mice to determine if gender differences were present. They determined that leukocyte invasion began one day following injury, and was greatly reduced on the fifth day post-injury in males, but persisted until the seventh day in female mice. Maximal leukocyte infiltration was seen to occur on the first day post-injury, and muscle sections obtained from male mice contained more fibers infiltrated by acid phosphatase-positive cells than muscle sections obtained from female mice. The difference seen between genders was suggested to be due to estrogen preventing an increase in macrophage concentrations in blood vessels by limiting the availability of endothelial cell adhesion molecules. They suggested that estrogen could decrease leukocyte migration into inflamed tissue because fewer endothelial cell adhesion molecules result in the failure of leukocytes to move out of the blood vessels and into the inflamed tissue. These results suggest that removal of damaged myofibers is slower in females than in males.

Tiidus[124] and Tiidus et al.,[121] have demonstrated that estrogen may protect skeletal muscle from muscle damage and inflammation, specifically neutrophil infiltration, following exercise through

inhibition and stabilization of calcium-activated calpains. Since it is believed that estrogen can act as a membrane stabilizer, it may act to minimize the disruption of the membrane during injury, which prevents the influx of calcium down its concentration gradient.[25] This would result in a decrease in calpain activity, and would prevent any further damage from occurring. Also, since muscle proteolysis by calpains attracts pro-inflammatory cells such as neutrophils, estrogen is suggested to protect muscle from further damage by inhibiting the recruitment of inflammatory leukocytes into muscle.[120] Tiidus et al.,[121] confirmed this hypothesis in an investigation that demonstrated a significant attenuation of 1-hour post exercise nuetrophil concentrations and myeloperoxidase activity in ovariectomized rats given estrogen supplementation. They also showed a reduction in calpain-like activity compared with ovariectomized rats treated with placebo. This study suggested that supplementation of estrogen, increased stability of the sarcolemma post-exercise, which prevented the activation of calpain.

Estrogen has also been suggested to influence pro-inflammatory cytokines; although, the relationship is complicated. Angstwurm et al.,[125] determined that during the follicular phase of the menstrual cycle, an increase in E2 was complemented with an increase in IL-6. Also, when progesterone levels rose following ovulation, a 1.5- to 4.4-fold decrease in plasma IL-6 was seen. Schwarz et al.,[126] demonstrated that male participants showed no difference in cytokine response between baseline samples and samples taken one to three weeks later. They also showed that TNF-α and IL-6 was significantly decreased in the luteal phase in pre-menopausal females, compared with their male counterparts, with the difference being more pronounced in females taking oral contraceptives. A diminished response during the luteal phase compared with the follicular phase was also seen. These results suggest that estrogen exhibits an inhibitory effect on activation of pro-inflammatory cytokines. However, Schwarz et al.,[126] also demonstrated a positive correlation between estradiol concentrations in plasma and the release of TNF-α and IL-6 following a challenge during the luteal phase.

Estrogen's influence on muscle regeneration

Little is known about the potential of estrogen to stimulate processes of regeneration of muscle such as satellite cell activation and proliferation. A study performed by McClung et al.,[127] reported re-growth and regeneration of skeletal muscle following a period of atrophy in rats is dependent on estrogen status. Also, differences in gender have been observed in satellite cell activation and proliferation. For example, Roth et al.,[128] demonstrated that, after nine weeks of resistance training, women showed a greater increase in the number of satellite cells in the vastuslateralis muscle than men. Tiidus et al.,[122] reported male rats that were supplemented with estrogen had an increase in satellite cells 72hours following a session of downhill running. In a follow-up study, they attempted to determine which stage(s) of the satellite cell cycle was influenced by estrogen. Following a similar protocol to the prior study, ovari ectomized female rats were supplemented with either estrogen or a placebo, and the researchers examined the histochemical changes in numbers of total satellite cells (Pax-7 positive), activated satellite cells (MyoD-positive), and proliferating satellite cells (BrdU-incorporated).[129] The results showed significant increases in all three of the observed markers. Therefore, they concluded that sex-mediated differences in muscle fiber regeneration and satellite cell numbers might be directly related to the influence of estrogen, and estrogen might have an influence on satellite cell activation in muscle following a bout of exercise through upstream mechanisms.

While the mechanisms by which estrogen influences satellite cell activation and proliferation are unknown, Enns & Tiidus[25] have suggested various receptor- and non-receptor-mediated roles for estrogen that may exist. Enns et al. showed that estrogen receptors play a critical role in influencing muscle repair processes through increases of satellite cell activation and proliferation. Blocking estrogen receptors was shown to completely eliminate both exercise- and estrogen-mediated increases in all three of the satellite cell markers.[96] Other data was able to pinpoint which estrogen receptor (ER-α) is specifically responsible for estrogens influence on satellite cells.[130]

It has also been suggested that, in the presence of estrogen, various downstream signaling pathways and targets of estrogen receptor binding exists that might be responsible for post-exercise up-regulation of satellite cells.[25] For example, Patten et al.,[131] and Sitnick et al.[132] demonstrated that through binding of estrogen to estrogen receptors, the PI3K/Akt pathway stimulates growth and protein synthesis. In addition, Kahlert et al.,[133] demonstrated that 17β-estradiol promotes cell growth via the estrogen receptor-mediated induction of the early genes *c-fos* and *egr-1* in myoblasts.

Fish oil and muscle damage

While the potential cyto-protective role of estrogen as an anti-oxidant has been of growing interest in the field, the use of fish oil as an anti-oxidant has been well established. Fish oil is part of the polyunsaturated fatty acids (PUFAs) family, and can be classified as omega-3 fatty acids (n-3) or omega-6 fatty acids (n-6) based on the location of the last double bond relative to the terminal methyl end of the molecule.[134] The precursors of n-3 and n-6 series of fatty acids are known as α-linolenic acid (ALA) and linoleic acid (LA), respectively. These are essential nutrients of the diet; however, the body is unable to produce these fatty acids.

Numerous studies have shown that n-3 attenuates the inflammatory response during muscle damage through the production of eicosanoids, which are mediators of inflammation, mainly through EPA and DHA. Dietary fish oil has been shown to result in a decrease in leukocyte chemotaxis, decreased production of ROS and other pro-inflammatory cytokines, and decreased adhesion molecule expression. The majority of studies involving fish oil supplementation have been focused on the protective effects of fish oil on the cardiovascular system; however, the possible effects of fish oil attenuating exercise-induced muscle damage is of growing interest. While the majority of studies on this subject have shown positive results, there are still some inconsistencies in the research, furthering the need for additional research in this area.

Overview of n-3 fatty acids

Fish oil derived n-3 fatty acids have been shown to have numerous benefits on health status that vary from cardiovascular benefits to slowing the progression of specific cancers.[135] PUFAs have also been shown to play a role in the regulation of the inflammatory response through the production of eicosanoids, which are inflammatory mediators.[136] Eicosanoids mediate the inflammatory response through production of pro-inflammatory and/or anti-inflammatory eicosanoids production. The production of pro-inflammatory eicosanoids prostaglandin E2 (PGE2), and leukotriene B4 (LTB4) are derived from the n-6 fatty acid arachidonic acid,[137] while the anti-inflammatory eicosanoids EPA and DHA are derived from n-3 PUFAs.[134]

Biochemically, n-3 fatty acids are a class of PUFAs that have the first carbon-carbon double bond in the third position from the methyl

Citation: McKinley-Barnard S, Willoughby DS. The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females. *J Nutr Health Food Eng.* 2015;2(6):205–219. DOI: 10.15406/jnhfe.2015.02.00082

end of the fatty acid, which is the n-3 position, with additional double bonds depending on the molecule. EPA is a longer chain n-3 fatty acid with a 20-carbon chain and 5 double bonds, and DHA with a 22-carbon chain with 6 double bonds. Both EPA and DHA are found in cold water fish that include salmon, mackerel, sardines, and herring. ALA is the shortest chain form of n-3 and only contains 18-carbon atoms with 3 double bonds, and can be found in plant sources such as flaxseeds, soybeans, and walnuts.[138] ALA can be converted into longer chain derivatives, and is therefore known as a precursor to other n-3 fatty acid chains. However, conversion from ALA to EPA and DHA is very inefficient and difficult to estimate an exact percentage,[139] with percentages ranging from 5-20% being converted to EPA and about 0.5-9% converted to DHA.[139,140]

Metabolism of ALA to EPA begins with the conversion of ALA to stearidonic acid, which is then elongated to eicosatetraenoic acid. The eicosatetraenoic acid formed is then converted to EPA, which is then either metabolized to DHA or to eicosanoids via enzymes cyclooxygenase (COX) and lipoxygenase (LOX). Conversion of EPA to DHA occurs with the addition of two carbons to form docosapentaenoic acid, the addition of two additional carbons to form tetracosapentaenoic acid, desaturation to form tetracosahexaenoic acid, and finally removal of two carbons by limited β-oxidation to produce DHA.

The pathway of elongation of n-6 and n-3 fatty acids occurs in the liver. Both LA and ALA are metabolized by the same enzymes, resulting in a competition between the LA and ALA; the one with an excess causes a decrease in metabolism of the other fatty acid.[134] Although, when the ratio of n-6 fatty acids and n-3 fatty acids is 1:1, the desaturases and elongases seem to exhibit an affinity to metabolize n-3 over n-6 fatty acids.[141,142] Usually, however, the ratio is in favor of n-6 fatty acids, which results in a greater conversion of LA to arachidonic acid.[143] Several studies have shown that increasing ALA intake to at least 4.5grams/day appears to produce a substantial increase in EPA plasma phospholipid content.[144–146] Decreasing LA in the diet has also been shown to increase metabolism of ALA to its longer chain derivatives.[147]

Inflammation and n-3 fatty acids

Eicosanoids include prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), and hydroxyeicosatetraenoic acids (HETE). These eicosanoids are derived primarily from 20-carbon PUFAs arachidonic acid and EPA, which are important inflammation regulators.[148] Specifically, these regulators control the intensity and duration of the inflammatory responses.[149] Although, the resulting physiological response depends on the types of cells present, the stimulus, the timing and concentration of eicosanoid production, and the sensitivity of the target cells.[150] As stated previously, there is a competition between n-6 and n-3 fatty acids since they utilize the same enzymes. This competition occurs at the enzymes COX and LOX.[134] Eicosanoids derived from n-6 are pro-inflammatory including PGE2, which induces production of IL-6 in macrophages and causes pain and vasodilation, and LTB4, which leads to the production of inflammatory cytokines such as TNF-α and IL-1 by macrophages.[148] Conversely, eicosanoids derived from n-3 are anti-inflammatory,[151] and act as a substrate for COX and LOX. This results in the production of the 3-series PGs and TXs, the 5-series LTs, and the hydroxyl-EPAs.[134]

Increased consumption of EPA and DHA has been shown in numerous studies to result in an increased concentration of those fatty acids in inflammatory cell phospholipids. An increased consumption of these fatty acids also has been shown in numerous studies to result in a decrease in the pro-inflammatory eicosanoids and an increase in anti-inflammatory eicosanoids. This suggests that the mediators that are formed from EPA and DHA are less potent than those mediators that are formed from arachidonic acid.[136] Several studies have identified a group of mediators that are formed from EPA and DHA by COX-2, termed E-series resolvins and D-series resolvins, respectively, also appear to have anti-inflammatory effects.[152,153]

There have been a few studies showing that n-3 fatty acid supplementation decreases ROS production, specifically H2O2 and superoxide, through stimulation of neutrophils. For example, Luostarinen & Saldeen[154] showed a significant decrease in superoxide generation by neutrophils without the involvement of COX or without altering neutrophil lysosomal enzyme release in 12 men that were supplemented with 5.4 grams of EPA and 3.2 grams of DHA daily for four weeks. Fisher et al.,[155] also showed that supplementation with 6 grams of EPA and DHA for 6 weeks decreased H2O2 production in monocytes. However, numerous studies that have utilized lower doses of EPA and DHA of less than 2.3 grams per day failed to demonstrate any effect on ROS production by neutrophils or monocytes.[156–158] Therefore, it is suggested that n-3 fatty acid supplementation may only be effective to attenuate ROS production at higher doses.

Long chain n-3 fatty acids exert another anti-inflammatory effect that is mediated at altered inflammatory gene expression through effects on transcription factors such as NF-Kb.[159] As stated previously, NF-κB is a transcription factor that plays a critical role in inflammatory signaling pathways. Several studies have shown that EPA inhibits the activity of NF-κB through a decrease in the degradation of IκB, which is an inhibitory subunit of NF-κB.[160–162]

n-3 supplementation and exercise

Numerous studies have hypothesized potential mechanisms by which n-3 supplementation may increase the benefits of exercise. One potential mechanism is by increasing lipolysis and β-oxidation. It has been suggested that n-3 fatty acids act as metabolic fuel partitioners[163] by up-regulating lipid oxidative enzymes and down-regulating lipogenic gene expression.[164,165] Several studies have claimed that this is in part due to the ability of n-3 fatty acids to bind and activate various peroxisome proliferator-activated receptor (PPAR) isoforms, which are members of the nuclear receptor superfamily.[166,167] Diep et al.,[168] determined that n-3 fatty acids have a higher affinity to act as ligands for the PPAR-α isoform, which is found in the nucleus of cells of many body tissues that exhibit high oxidative rates of fatty acids, including skeletal muscle. Theoretically, an increase in the activity of PPAR-α should facilitate an increase in the reliance on fat as a fuel source during exercise, thus sparing muscle glycogen and improving performance.[138]

Another mechanism in which n-3 fatty acid supplementation may increase the benefits of exercise is by increasing fatty acid delivery to the working muscles through an increase in blood flow.[169] It has been suggested that this improvement in blood flow is due to n-6 eicosanoid production being suppressed by n-3 fatty acids, leading to a decrease in pro-inflammatory eicosanoids, which cause vasoconstriction and an increase in anti-inflammatory eicosanoids, which allows for vasodilation.[138] It has also been suggested that n-3 fatty acids supplementation may prevent red blood cells from deformity, which may be attributed to an increase in the peroxidation of lipid membranes that occurs as a result of ROS production.[170]

Fish oil and eccentric exercise

Fish oil supplementation has been widely studied during aerobic exercise as well as in various disease states; comparatively, there are very few studies that focus on fish oil supplementation and the effects on eccentric exercise and inflammation. As stated previously, muscle weakness and soreness are common occurrences of EIMD. Research has shown that following a quick recovery within 2 to 3 hours following exercise, muscle strength slowly returns to baseline, but may remain depressed for a few days or weeks, depending on the degree of damage.[171,172] During this time, structural damage and subsequent inflammatory events may progress before muscle repair and regeneration occurs. EIMD is usually accompanied by DOMS, but the mechanisms are not fully understood. Phillips et al.,[173] determined that DHA supplementation reduced the exercise-induced inflammatory response that occurs following eccentric exercise. Tartibian et al.,[174] hypothesized that the supplementation of n-3 fatty acids would result in an anti-inflammatory response to exercise, which may subsequently reduce DOMS. They proved their hypothesis correct by demonstrating that 1.8 grams of n-3 fatty acid supplementation in healthy males reduced pro-inflammatory eicosanoids such as IL-6, PGE2, and TNF-α following eccentric exercise. In a previous study, Tartibian et al.,[34] showed that ingestion of 1.8 grams of n-3 fatty acids decreased DOMS in men following eccentric exercise. More recently, Lembke et al.,[175] showed that supplementation of 2.7grams of n-3 fatty acids for 30 days could reduce DOMS and C-reactive protein (CRP) following eccentric exercise when compared to sunflower oil. Jouris et al.,[35] determined that following ingestion of 3 grams of n-3 fatty acids for 7days, DOMS was significantly reduced in 3 males and 8 females following eccentric exercise. Considering the results of these studies, Kim & Lee[176] suggested that an amount of 1.8 to 3 grams should be sufficient to effectively reduce DOMS following eccentric exercise.

Several studies have also measured oxidative stress markers to explain the potential reason n-3 fatty acids reduce DOMS, but the results have been inconsistent among the studies. For example, one study determined that 1.8grams of n-3 fatty acid supplementation for 30days did not significantly reduce DOMS and malondialdehyde (MDA) following exercise,[26] while another study reported that 3 grams of n-3 fatty acid ingestion for 6 weeks significantly reduced thiobarbituric acid-reactive substance (TBARS), which is a marker of lipid peroxidation, when compared to a placebo.[36] However, Gray et al.,[36] did not determine that there was a difference in DOMS between the experimental group and the placebo group. These results suggest that n-3 supplementation is more associated with the inflammatory response as opposed to with oxidative stress for decreasing DOMS following eccentric exercise.

Conclusion

While the exact mechanisms of EIMD, and how estrogen and fish oil may potentially play a protective role in the process of muscle damage has yet to be clearly elucidated, this area of research is becoming of greater interest to researchers. More research needs to be conducted to objectively determine the exact mechanisms of EIMD, and how muscle damage may potentially be attenuated either with estrogen or other anti-oxidants such as fish oil. The hypotheses that have been suggested thus far have shown promise, with the majority of researcher data supporting their claims; however, more research must been done to be able to confidently confirm them. Estrogen and fish oil are thought to attenuate EIMD mainly during the inflammatory phase of muscle damage. However, the inflammatory process may be necessary to allow muscle to adapt, regenerate, and repair. Further research must be conducted to determine if this is indeed the case. While this area is of growing interest, very little is concretely known about these processes and mechanisms, and further research is obviously warranted to determine the exact mechanisms that are involved.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

References

1. Armstrong RB, Warren GL, Warren JA. Mechanisms of exercise-induced muscle fibre injury. *Sports Med*. 1991;12(3):184–207.
2. Enoka RM. Eccentric contractions require unique activation strategies by the nervous system. *J Appl Physiol*. 1996;81(6):2339–2346.
3. Clarkson PM, Newham DJ. Associations between muscle soreness, damage, and fatigue. *Adv Exp Med Biol*. 1995;384:457–469.
4. Kenney LW, Wilmore J, Costill D. Physiology of Sport and Exercise. 6th ed. *Human Kinetics*. 2015.
5. Beltman JGM, van der Vliet MR, Sargeant AJ, et al. Metabolic cost of lengthening, isometric and shortening contractions in maximally stimulated rat skeletal muscle. Acta Physiol Scand. 2004;182(2):179–187.
6. MacIntosh BR, Gardiner PF, McComas AJ. Skeletal Muscle: Form and Function. *Human Kinetics*. 2006.
7. Howell JN, Chleboun G, Conatser R. Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *J Physiol.* 1993;464:183–196.
8. Cleak MJ, Eston RG. Delayed onset muscle soreness: mechanisms and management. *J Sports Sci.* 1992;10(4):325–341.
9. Eston RG, Finney S, Baker S, et al. Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise. *J Sports Sci*. 1996;14:291–299.
10. Clarkson PM, Nosaka K, Braun B. Muscle function after exercise-induced muscle damage and rapid adaptation. *Med Sci Sports Exerc*. 1992;24(5):512–520.
11. Child RB, Saxton JM, Donnelly AE. Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. *J Sports Sci*. 1998;16(4):301–308.
12. Kendall B, Eston R. Exercise-induced muscle damage and the potential protective role of estrogen. *Sports Med.* 2002;32(2):103–123.
13. Cheung K, Hume P, Maxwell L. Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med.* 2003;33(2):145–164.
14. Pyne DB. Regulation of neutrophil function during exercise. *Sports Med*. 1994;17(4):245–258.
15. Dudley GA, Tesch PA, Miller BJ, et al. Importance of eccentric actions in performance adaptations to resistance training. *Aviat Space Environ Med*. 1991;62(6):543–550.
16. Hortobágyi T, Hill JP, Houmard JA, et al. Adaptive responses to muscle lengthening and shortening in humans. *J Appl Physiol.* 1996;80(3):765–772.

17. Hather BM, Tesch PA, Buchanan P, et al. Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiol Scand.* 1991;143(2):177–185.

18. Higbie EJ, Cureton KJ, Warren GL, et al. Effects of concentric and eccentric training on muscle strength, cross-sectional area, and neural activation. *J Appl Physiol (1985)*. 1996;81:2173–2181.

19. Adams GR, Cheng DC, Haddad F, et al. Skeletal muscle hypertrophy in response to isometric, lengthening, and shortening training bouts of equivalent duration. *J Appl Physiol (1985)*. 2004;96(5):1613–1618.

20. Hortobágyi T, Barrier J, Beard D, et al. Greater initial adaptations to submaximal muscle lengthening than maximal shortening. *J Appl Physiol.* 1996;81(4):1677–1682.

21. Lastayo PC, Reich TE, Urquhart M, et al. Chronic eccentric exercise: improvements in muscle strength can occur with little demand for oxygen. *Am J Physiol.* 1999;276(2 Pt 2):R611–R615.

22. Hortobagyi T, Money J, Zheng D, et al. Muscle adaptations to 7days of exercise in young and older humans: eccentric overload versus standard resistance training. *J Aging Phys Act*. 2002;10(3):290–305.

23. Yu J-G, Fürst DO, Thornell LE. The mode of myofibril remodelling in human skeletal muscle affected by DOMS induced by eccentric contractions. *Histochem Cell Biol*. 2003;119(5):383–393.

24. Yu J-G, Carlsson L, Thornell L-E. Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. *Histochem Cell Biol*. 2004;121(3):219–227.

25. Enns DL, Tiidus PM. The influence of estrogen on skeletal muscle: sex matters. *Sports Med*. 2010;40(1):41–58.

26. Lenn J, Uhl T, Mattacola C, et al. The effects of fish oil and isoflavones on delayed onset muscle soreness. *Med Sci Sports Exerc.* 2002;34(10):1605–1613.

27. Lopez-Huertas E. The effect of EPA and DHA on metabolic syndrome patients: a systematic review of randomised controlled trials. *Br J Nutr.* 2012;107(2):S185–S194.

28. Beavers KM, Serra MC, Beavers DP, et al. Soymilk supplementation does not alter plasma markers of inflammation and oxidative stress in postmenopausal women. *Nutr.* 2009;29(9):616–622.

29. Buford TW, Cooke MB, Manini TM, et al. Effects of age and sedentary lifestyle on skeletal muscle NF-kappaB signaling in men. *J Gerontol A BiolSci Med Sci.* 2010;65(5):532–537.

30. Kerksick CM, Kreider RB, Willoughby DS. Intramuscular adaptations to eccentric exercise and antioxidant supplementation. *Amino Acids.* 2010;39(1):219–232.

31. McKinley S, Willoughby DS. Effectiveness of antioxidant nutraceuticals in attenuating canonical NF-κB signaling in human skeletal muscle resulting from exercise-induced inflammation and oxidative stress. *J Nutr Health Food Eng*. 2014;1(6):1–8.

32. Kerksick C, Taylor L, Harvey A, et al. Gender-related differences in muscle injury, oxidative stress, and apoptosis. *Med Sci Sports Exerc.* 2008;40(10):1772–1780.

33. Willoughby DS, Taylor M, Taylor L. Glucocorticoid receptor and ubiquitin expression after repeated eccentric exercise. *Med Sci Sports Exerc*. 2003;35(12):2023–2031.

34. Tartibian B, Maleki BH, Abbasi A. The effects of ingestion of omega-3 fatty acids on perceived pain and external symptoms of delayed onset muscle soreness in untrained men. *Clin J Sport Med*. 2009;19(2):115–119.

35. Jouris KB, McDaniel JL, Weiss EP. The Effect of Omega-3 Fatty Acid Supplementation on the Inflammatory Response to eccentric strength exercise. *J Sports Sci Med*. 2011;10(3):432–438.

36. Gray P, Chappell A, Jenkinson AM, et al. Fish oil supplementation reduces markers of oxidative stress but not muscle soreness after eccentric exercise. *Int J Sport Nutr Exerc Metab*. 2014;24(2):206–214.

37. Bloomer RJ, Larson DE, Fisher-Wellman KH, et al. Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: a randomized, placebo controlled, cross-over study. *Lipids Health Dis*. 2009;8:36.

38. Houghton D, Onambele GL. Can a standard dose of eicosapentaenoic acid (EPA) supplementation reduce the symptoms of delayed onset of muscle soreness? *J Int Soc Sports Nutr*. 2012;9(1):2.

39. Armstrong RB. Initial events in exercise-induced muscular injury. *Med Sci Sports Exerc*. 1990;22(4):429–435.

40. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol.* 2001;537(Pt 2):333–345.

41. Best TM, Fiebig R, Corr DT, et al. Free radical activity, antioxidant enzyme, and glutathione changes with muscle stretch injury in rabbits. *J Appl Physiol (1985)*. 1999;87(1):74–82.

42. Byrd SK. Alterations in the sarcoplasmic reticulum: a possible link to exercise-induced muscle damage. *Med Sci Sports Exerc.* 1992;24(5):531–536.

43. McArdle A, Jackson M. Intracellular mechanisms involved in skeletal muscle damage. *Muscle Damage*. Oxford: Oxford University Press; 1997.

44. Amelink GJ, Van der Kallen CJ, Wokke JH, et al. Dantrolene sodium diminishes exercise-induced muscle damage in the rat. *Eur J Pharmacol.* 1990;179(1–2):187–192.

45. Ingalls CP, Warren GL, Williams JH, et al. E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *J Appl Physiol*. 1998;85(1):58–67.

46. Clarkson PM, Sayers SP. Etiology of exercise-induced muscle damage. *Can J Appl Physiol.* 1999;24(3):234–248.

47. Murachi T, Tanaka K, Hatanaka M, et al. Intracellular Ca2+-dependent protease (calpain) and its high-molecular-weight endogenous inhibitor (calpastatin). *Adv Enzyme Regul.* 1980;19:407–424.

48. Mellgren RL. Calcium-dependent proteases: an enzyme system active at cellular membranes? *FASEB*. 1987;1(2):110–115.

49. Belcastro AN, Shewchuk LD, Raj DA. Exercise-induced muscle injury: a calpain hypothesis. *Mol Cell Biochem*. 1998;179(1–2):135–145.

50. Bullard B, Sainsbury G, Miller N. Digestion of proteins associated with the Z-disc by calpain. *J Muscle Res Cell Motil.* 1990;11(3):271–279.

51. Nielsen TB, Field JB, Dedman JR. Association of calmodulin with lysosomes. *J Cell Sci*. 1987;87(Pt 2):327–336.

52. Rodemann HP, Waxman L, Goldberg AL. The stimulation of protein degradation in muscle by Ca2+ is mediated by prostaglandin E2 and does not require the calcium-activated protease. *J Biol Chem*. 1982;257(15):8716–8723.

53. Vusse G van der, Bilsen M van, Reneman RS. Is Phospholipid Degradation a Critical Event in Ischemia-and Reperfusion-Induced Damage? *Physiology.* 1989;4(2):49–53.

54. Evans WJ, Cannon JG. The metabolic effects of exercise-induced muscle damage. *Exerc Sport Sci Rev*. 1991;19:99–125.

55. MacIntyre DL, Reid WD, McKenzie DC. Delayed muscle soreness. The inflammatory response to muscle injury and its clinical implications. *Sports Med.* 1995;20(1):24–40.

56. Tidball JG. Inflammatory cell response to acute muscle injury. *Med Sci Sports Exerc.* 1995;27(7):1022–1032.

Citation: McKinley-Barnard S, Willoughby DS. The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females. *J Nutr Health Food Eng.* 2015;2(6):205–219. DOI: 10.15406/jnhfe.2015.02.00082

57. Imura H, Fukata J, Mori T. Cytokines and endocrine function: an interaction between the immune and neuroendocrine systems. *Clin Endocrinol.* 1991;35(2):107–115.

58. Cantini M, Carraro U. Macrophage-released factor stimulates selectively myogenic cells in primary muscle culture. *J Neuropathol Exp Neurol*. 1995;54(1):121–128.

59. Merly F, Lescaudron L, Rouaud T, et al. Macrophages enhance muscle satellite cell proliferation and delay their differentiation. *Muscle Nerve.* 1999;22(6):724–732.

60. Lescaudron L, Peltékian E, Fontaine-Pérus J, et al. Blood borne macrophages are essential for the triggering of muscle regeneration following muscle transplant. *Neuromuscul Disord.* 1999;9(2):72–80.

61. Pingitore A, Lima GPP, Mastorci F, et al. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. *Nutr.* 2015;31(7–8):916–922.

62. Hayden MS, West AP, Ghosh S. SnapShot: NF-kappaB signaling pathways. *Cell*. 2006;127(6):1286–1287.

63. Nikolaidis MG, Jamurtas AZ, Paschalis V, et al. The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations. *Sports Med.* 2008;38(7):579–606.

64. Ji LL, Gomez-Cabrera M-C, Vina J. Role of nuclear factor kappaB and mitogen-activated protein kinase signaling in exercise-induced antioxidant enzyme adaptation. *Appl Physiol Nutr Metab Physiol*. 2007;32(5):930–935.

65. Smolka MB, Zoppi CC, Alves AA, et al. HSP72 as a complementary protection against oxidative stress induced by exercise in the soleus muscle of rats. *Am J Physiol Regul Integr Comp Physiol*. 2000;279(5):R1539–R1545.

66. Brotto MA, Nosek TM. Hydrogen peroxide disrupts Ca2+ release from the sarcoplasmic reticulum of rat skeletal muscle fibers. *J Appl Physiol (1985).* 1996;81(2):731–737.

67. Radák Z, Kaneko T, Tahara S, et al. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. *Free Radic Biol Med*. 1999;27(1–2):69–74.

68. Ji LL. Antioxidant signaling in skeletal muscle: a brief review. *Exp Gerontol*. 2007;42(7):582–593.

69. Allen RG, Tresini M. Oxidative stress and gene regulation. *Free RadicBiol Med*. 2000;28(3):463–499.

70. Lander HM. An essential role for free radicals and derived species in signal transduction. *FASEB J*. 1997;11(2):118–124.

71. Jackson MJ, Papa S, Bolaños J, et al. Antioxidants, reactive oxygen and nitrogen species, gene induction and mitochondrial function. *Mol Aspects Med.* 2002;23(1–3):209–285.

72. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev.* 2008;88(4):1243–1276.

73. Moran LK, Gutteridge JM, Quinlan GJ. Thiols in cellular redox signalling and control. *Curr Med Chem*. 2001;8(7):763–772.

74. Hansen JM, Zhang H, Jones DP. Differential oxidation of thioredoxin-1, thioredoxin-2, and glutathione by metal ions. *Free Radic Biol Med*. 2006;40(1):138–145.

75. Peterson JM, Bakkar N, Guttridge DC. NF-κB signaling in skeletal muscle health and disease. *Curr Top Dev Biol.* 2011;96:85–119.

76. Bakkar N, Guttridge DC. NF-kappaB signaling: a tale of two pathways in skeletal myogenesis. *Physiol Rev*. 2010;90(2):495–511.

77. Li H, Malhotra S, Kumar A. Nuclear factor-kappa B signaling in skeletal muscle atrophy. *J Mol Med (Berl)*. 2008;86(10):1113–1126.

78. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*. 1999;18(49):6853–6866.

79. Li YP, Schwartz RJ, Waddell ID, et al. Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-kappaB activation in response to tumor necrosis factor alpha. *FASEB J.* 1998;12(10):871–880.

80. Kramer HF, Goodyear LJ. Exercise, MAPK, and NF-kappaB signaling in skeletal muscle. *J Appl Physiol (1985).* 2007;103(1):388–395.

81. Solt LA, May MJ. The IkappaB kinase complex: master regulator of NF-kappaB signaling. *Immunol Res*. 2008;42(1–3):3–18.

82. Jackman RW, Kandarian SC. The molecular basis of skeletal muscle atrophy. *Am J Physiol Cell Physiol.* 2004;287(4):C834–C843.

83. Bhatnagar S, Panguluri SK, Gupta SK, et al. Tumor necrosis factor-α regulates distinct molecular pathways and gene networks in cultured skeletal muscle cells. *PloS One*. 2010;5(10):e13262.

84. Mourkioti F, Rosenthal N. NF-kappaB signaling in skeletal muscle: prospects for intervention in muscle diseases. *J Mol Med (Berl)*. 2008;86(7):747–759.

85. Kumar A, Takada Y, Boriek AM, et al. Nuclear factor-kappaB: its role in health and disease. *J Mol Med (Berl)*. 2004;82(7):434–448.

86. Mourkioti F, Kratsios P, Luedde T, et al. Targeted ablation of IKK2 improves skeletal muscle strength, maintains mass, and promotes regeneration. *J Clin Invest.* 2006;116(11):2945–2954.

87. Sen CK, Khanna S, Reznick AZ, et al. Glutathione regulation of tumor necrosis factor-alpha-induced NF-kappa B activation in skeletal muscle-derived L6 cells. *BiochemBiophys Res Commun*. 1997;237(3):645–649.

88. Bar-Shai M, Carmeli E, Ljubuncic P, et al. Exercise and immobilization in aging animals: the involvement of oxidative stress and NF-kappaB activation. *Free RadicBiol Med*. 2008;44(2):202–214.

89. Lugrin J, Rosenblatt-Velin N, Parapanov R, et al. The role of oxidative stress during inflammatory processes. *Biol Chem*. 2014;395(2):203–230.

90. Carroll MP, May WS. Protein kinase C-mediated serine phosphorylation directly activates Raf-1 in murine hematopoietic cells. *J Biol Chem.* 1994;269(2):1249–1256.

91. Bunt JC. Metabolic actions of estradiol: significance for acute and chronic exercise responses. *Med Sci Sports Exerc.* 1990;22(3):286–290.

92. Katzenellenbogen BS, Montano MM, Le Goff P, et al. Antiestrogens: mechanisms and actions in target cells. *J Steroid Biochem Mol Biol.* 1995;53(1–6):387–393.

93. Tiidus PM. Can estrogens diminish exercise induced muscle damage? *Can J Appl Physio*l. 1995;20(1):26–38.

94. Gruber DM, Huber JC. Conjugated estrogens--the natural SERMs. *Gynecol Endocrinol*. 1993;13(Suppl 6):9–12.

95. Strehlow K, Rotter S, Wassmann S, et al. Modulation of antioxidant enzyme expression and function by estrogen. *Circ Res.* 2003;93(2):170–177.

96. Enns DL, Iqbal S, Tiidus PM. Oestrogen receptors mediate oestrogen-induced increases in post-exercise rat skeletal muscle satellite cells. *Acta Physiol* (Oxf). 2008;194(1):81–93.

97. Karlsson J. *Antioxidants and Exercise*. 1st ed. IL: Human Kinetics, Champaign, USA; 1997.

98. Yagi K, Komura S. Inhibitory effect of female hormones on lipid peroxidation. *Biochem Int*. 1986;13(6):1051–1055.

99. Sugioka K, Shimosegawa Y, Nakano M. Estrogens as natural antioxidants of membrane phospholipid peroxidation. *FEBS Lett.* 1987;210(1):37–39.

100. Huber LA, Scheffler E, Poll T, et al. 17 beta-estradiol inhibits LDL oxidation and cholesteryl ester formation in cultured macrophages. *Free Radic Res Commun*. 1990;8(3):167–173.

Citation: McKinley-Barnard S, Willoughby DS. The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females. *J Nutr Health Food Eng.* 2015;2(6):205–219. DOI: 10.15406/jnhfe.2015.02.00082

101. Mooradian AD. Antioxidant properties of steroids. *J Steroid Biochem Mol Biol.* 1993;45(6):509–511.

102. Subbiah MT, Kessel B, Agrawal M, et al. Antioxidant potential of specific estrogens on lipid peroxidation. *J Clin Endocrinol Metab*. 1993;77(4):1095–1097.

103. Ayres S, Tang M, Subbiah MT. Estradiol-17beta as an antioxidant: some distinct features when compared with common fat-soluble antioxidants. *J Lab Clin Med*. 1996;128(4):367–375.

104. Bär PR, Amelink GJ. Protection against muscle damage exerted by oestrogen: hormonal or antioxidant action? *Biochem Soc Trans.* 1997;25(1):50–54.

105. Ayres S, Abplanalp W, Liu JH, et al. Mechanisms involved in the protective effect of estradiol-17beta on lipid peroxidation and DNA damage. *Am J Physiol.* 1998;274(6 Pt 1):E1002–E1008.

106. Persky AM, Green PS, Stubley L, et al. Protective effect of estrogens against oxidative damage to heart and skeletal muscle in vivo and in vitro. *Proc Soc Exp Biol Med.* 2000;223(1):59–66.

107. Paroo Z, Dipchand ES, Noble EG. Estrogen attenuates postexercise HSP70 expression in skeletal muscle. *Am J Physiol Cell Physiol.* 2002;282(2):C245–C251.

108. Feng X, Li G, Wang S. Effects of estrogen on gastrocnemius muscle strain injury and regeneration in female rats. *Acta Pharmacol Sin*. 2004;25(1):1489–1494.

109. Stupka N, Tiidus PM. Effects of ovariectomy and estrogen on ischemia-reperfusion injury in hindlimbs of female rats. *J Appl Physiol* 91(4):1828–1835.

110. Tiidus PM, Bombardier E, Hidiroglou N, et al. Estrogen administration, postexercise tissue oxidative stress and vitamin C status in male rats. *Can J Physiol Pharmacol.* 1998;76(10–11):952–960.

111. Dernbach AR, Sherman WM, Simonsen JC, et al. No evidence of oxidant stress during high-intensity rowing training. *J Appl Physiol*. 1993;74(5):2140–2145.

112. Ayres S, Baer J, Subbiah MT. Exercised-induced increase in lipid peroxidation parameters in amenorrheic female athletes. *Fertil Steril.* 1998;69(1):73–77.

113. Wiseman H, Quinn P. The antioxidant action of synthetic oestrogens involves decreased membrane fluidity: relevance to their potential use as anticancer and cardioprotective agents compared to tamoxifen? *Free Radic Res*. 1994;21(3):187–194.

114. Yoshikawa T, Yoshida N. Vitamin E and leukocyte-endothelial cell interactions. *Antioxid Redox Signal*. 2000;2(4):821–825.

115. Baird MF, Graham SM, Baker JS, et al. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *J Nutr Metab.* 2012;2012:960363.

116. Saks V. The phosphocreatine-creatine kinase system helps to shape muscle cells and keep them healthy and alive. *J Physiol*. 2008;586(Pt 12):2817–2818.

117. Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med.* 1999;27(1):43–59.

118. Amelink GJ, Bär PR. Exercise-induced muscle protein leakage in the rat. Effects of hormonal manipulation. *J Neurol Sci.* 1986;76(1):61–68.

119. Amelink GJ, Koot RW, Erich WB, et al. Sex-linked variation in creatine kinase release, and its dependence on oestradiol, can be demonstrated in an in-vitro rat skeletal muscle preparation. *Acta Physiol Scand*. 1990;138(2):115–124.

120. Tiidus PM, Bombardier E. Oestrogen attenuates post-exercise myeloperoxidase activity in skeletal muscle of male rats. *Acta Physiol Scand*. 1999;166(2):85–90.

121. Tiidus PM, Holden D, Bombardier E, et al. Estrogen effect on post-exercise skeletal muscle neutrophil infiltration and calpain activity. *Can J Physiol Pharmacol*. 2001;79(5):400–406.

122. Tiidus PM, Deller M, Liu XL. Oestrogen influence on myogenic satellite cells following downhill running in male rats: a preliminary study. *Acta Physiol Scand*. 2005;184(1):67–72.

123. St Pierre Schneider B, Correia LA, et al. Sex differences in leukocyte invasion in injured murine skeletal muscle. *Res Nurs Health*. 1999;22(3):243–250.

124. Tiidus PM. Influence of estrogen on skeletal muscle damage, inflammation, and repair. *Exerc Sport Sci Rev*. 2003;31(1):40–44.

125. Angstwurm MW, Gärtner R, Ziegler-Heitbrock HW. Cyclic plasma IL-6 levels during normal menstrual cycle. *Cytokine.* 1997;9(5):370–374.

126. Schwarz E, Schäfer C, Bode JC, et al. Influence of the menstrual cycle on the LPS-induced cytokine response of monocytes. *Cytokine.* 2000;12(4):413–416.

127. McClung JM, Davis JM, Wilson MA, et al. Estrogen status and skeletal muscle recovery from disuse atrophy. *J Appl Physiol*. 2006;100(6):2012–2023.

128. Roth SM, Martel GF, Ivey FM, et al. Skeletal muscle satellite cell characteristics in young and older men and women after heavy resistance strength training. *J Gerontol A Biol Sci Med Sci*. 2001;56(6):B240–B247.

129. Enns DL, Tiidus PM. Estrogen influences satellite cell activation and proliferation following downhill running in rats. *J Appl Physiol.* 2008;104(2):347–353.

130. Thomas A, Bunyan K, Tiidus PM. Oestrogen receptor-alpha activation augments post-exercise myoblast proliferation. *Acta Physiol*. 2010;198(1):81–89.

131. Patten RD, Pourati I, Aronovitz MJ, et al. 17beta-estradiol reduces cardiomyocyte apoptosis in vivo and in vitro via activation of phospho-inositide-3 kinase/Akt signaling. *Circ Res.* 2004;95(7):692–699.

132. Sitnick M, Foley AM, Brown M, et al. Ovariectomy prevents the recovery of atrophied gastrocnemius skeletal muscle mass. *J Appl Physiol.* 2006;100(1):286–293.

133. Kahlert S, Grohé C, Karas RH, et al. Effects of estrogen on skeletal myoblast growth. *Biochem Biophys Res Commun.* 1997;232(2):373–378.

134. Wall R, Ross RP, Fitzgerald GF, et al. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev.* 2010;68(5):280–289.

135. Siriwardhana N, Kalupahana NS, Moustaid-Moussa N. Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. *Adv Food Nutr Res*. 2012;65:211–222.

136. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr.* 2006;83(6 Suppl):1505S–1519S.

137. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr.* 2000;71(1 Suppl):343S–348S.

138. Tiryaki-Sönmez G, Schoenfeld B, Vatansever-Ozen S. Omega-3 fatty acids and exercise: a review of their combined effects on body composition and physical performance. *Biomed Hum Kine*t. 2011;3(3):23–29.

139. Plourde M, Cunnane SC. Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. *Appl Physiol Nutr Metab Physiol.* 2007;32(4):619–634.

140. Burdge GC. Metabolism of alpha-linolenic acid in humans. *Prostaglandins Leukot Essent Fatty Acids*. 2007;75(3):161–168.

141. Das UN. Biological significance of essential fatty acids. *J Assoc Physicians India*. 2006;54:309–319.

142. Simopoulos AP. Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. *World Rev Nutr Diet*. 2003;92:1–22.

143. Simopoulos AP. n-3 fatty acids and human health: defining strategies for public policy. *Lipids*. 2001;36(Suppl):S83–S89.

144. Mantzioris E, James MJ, Gibson RA, et al. Dietary substitution with an alpha-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *Am J Clin Nutr*. 1994;59(6):1304–1309.

145. Wallace FA, Miles EA, Calder PC. Comparison of the effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. *Br J Nutr.* 2003;89(5):679–689.

146. Finnegan YE, Minihane AM, Leigh-Firbank EC, et al. Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. *Am J Clin Nutr*. 2003;77(4):783–795.

147. Liou YA, King DJ, Zibrik D, et al. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *J Nutr*. 2007;137(4):945–952.

148. Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest*. 2001;108(1):15–23.

149. Kinsella JE, Lokesh B, Broughton S, et al. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutr.* 1990;6(1):24–44.

150. Calder PC. Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Mol Nutr Food Res.* 2008;52(8):885–897.

151. Bagga D, Wang L, Farias-Eisner R, et al. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proc Natl Acad Sci U. S. A*. 2003;100(4):1751–1756.

152. Serhan CN, Clish CB, Brannon J, et al. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med.* 2000;192(8):1197–1204.

153. Hong S, Gronert K, Devchand PR, et al. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J Biol Chem*. 2003;278(17):14677–14687.

154. Luostarinen R, Saldeen T. Dietary fish oil decreases superoxide generation by human neutrophils: relation to cyclooxygenase pathway and lysosomal enzyme release. *Prostaglandins Leukot Essent Fatty Acids*. 1996;55(3):167–172.

155. Fisher M, Levine PH, Weiner BH, et al. Dietary n-3 fatty acid supplementation reduces superoxide production and chemiluminescence in a monocyte-enriched preparation of leukocytes. *Am J Clin Nutr.* 1990;51(5):804–808.

156. Thies F, Miles EA, Nebe-von-Caron G, et al. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids*. 2001;36(11):1183–1193.

157. Kew S, Banerjee T, Minihane AM, et al. Lack of effect of foods enriched with plant- or marine-derived n-3 fatty acids on human immune function. *Am J Clin Nutr.* 2003;77(5):1287–1295.

158. Miles EA, Banerjee T, Dooper MMBW, et al. The influence of different combinations of gamma-linolenic acid, stearidonic acid and EPA on immune function in healthy young male subjects. *Br J Nutr*. 2004;91(6):893–903.

159. Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc*. 2002;61(3):345–358.

160. Lo CJ, Chiu KC, Fu M, et al. Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NF kappa B activity. *J Surg Res.* 1999;82(2):216–221.

161. Novak TE, Babcock TA, Jho DH, et al. NF-kappa B inhibition by omega -3 fatty acids modulates LPS-stimulated macrophage TNF-alpha transcription. *Am J Physiol Lung Cell Mol Physiol.* 2003;284:L84–L89.

162. Zhao Y, Joshi-Barve S, Barve S, et al. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. *J Am Coll Nutr.* 2004;23(1):71–78.

163. Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *J Nutr.* 2001;131(4):1129–1132.

164. Jump DB, Clarke SD, Thelen A, et al. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J Lipid Res*. 1994;35(6):1076–1084.

165. Raclot T, Groscolas R, Langin D, et al. Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues. *J Lipid Res.* 1997;38(10):1963–1972.

166. Lin Q, Ruuska SE, Shaw NS, et al. Ligand selectivity of the peroxisome proliferator-activated receptor alpha. *Biochemistry*. 1999;38(1):185–190.

167. Neschen S, Morino K, Dong J, et al. n-3 Fatty acids preserve insulin sensitivity in vivo in a peroxisome proliferator-activated receptor-alpha-dependent manner. *Diabetes*. 2007;56(4):1034–1041.

168. Diep QN, Touyz RM, Schiffrin EL. Docosahexaenoic acid, a peroxisome proliferator-activated receptor-alpha ligand, induces apoptosis in vascular smooth muscle cells by stimulation of p38 mitogen-activated protein kinase. *Hypertension*. 2000;36(5):851–855.

169. Hill AM, Buckley JD, Murphy KJ, et al. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. *Am J Clin Nutr*. 2007;85(5):1267–1274.

170. Szygula Z. Erythrocytic system under the influence of physical exercise and training. *Sports Med.* 1990;10(3):181–197.

171. Paulsen G, Crameri R, Benestad HB, et al. Time course of leukocyte accumulation in human muscle after eccentric exercise. *Med Sci Sports Exerc.* 2010;42(1):75–85.

172. Paulsen G, Mikkelsen UR, Raastad T, et al. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev.* 2012;18:42–97.

173. Phillips T, Childs AC, Dreon DM, et al. A dietary supplement attenuates IL-6 and CRP after eccentric exercise in untrained males. *Med Sci Sports Exerc.* 2003;35(12):2032–2037.

174. Tartibian B, Maleki BH, AbbasiA. Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men. *Clin J Sport Med.* 2011;21(2):131–137.

175. Lembke P, Capodice J, Hebert K, et al. Influence of omega-3 (n3) index on performance and wellbeing in young adults after heavy eccentric exercise. *J Sports Sci Med.* 2014;13(1):151–156.

176. Kim J, Lee J. A review of nutritional intervention on delayed onset muscle soreness. Part I. *J Exerc Rehabil*. 2014;10(6):349–356.

Citation: McKinley-Barnard S, Willoughby DS. The potential cytoprotective influence of estradiol and fish oil supplementation on indices of exercise-induced muscle damage in females. *J Nutr Health Food Eng.* 2015;2(6):205–219. DOI: 10.15406/jnhfe.2015.02.00082