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# Nutritional Interventions as Potential Strategy to Minimize Exercise-Induced Muscle Injuries in Sports

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## 1. Introduction

Muscle injury has been related to resistance exercise and prolonged endurance exercise paradigms both leading to significant local mechanical constraints followed by focal disorders such as sarcolemmal damage and leakage of intracellular proteins, oedema, myofibrillar disorganization and microtrauma-triggered inflammation. These unfavorable events lead to variable soreness, swelling, loss in muscle strength and function with reduced range of motion.

To date strategies finalized to minimize exercise-induced muscle injury are scarce and often not adequately supported by research studies.

Based on the notion that dietary supplementations may exert a variety of beneficial effects on the skeletal muscle, in the last 20 years there has been a great deal of interest in nutritional strategies aiming to attenuate signs and symptoms of exercise induced muscle injuries. Anyhow a large number of variables influences the muscular outcome of nutritional supplements, strongly depending on nutrient type, genotype, age, and regulation of nutrient sensing pathways.

Overall there is a paucity of studies on the topic, partly related to the high number of supplements to be considered and their combined use. In general nutrients as vitamins (as vitamin C), N-acetyl-cysteine, L-carnitine, creatine, and branched chain amino acids (BCAA) may exert a potential beneficial role but the underlying cellular mechanism, the optimal dosage and the duration of the pretreatment/treatment period are currently unknown.

This chapter addresses the current knowledge on the potential use of nutritional supplements in preventing and/or minimizing muscle injuries due to resistance or endurance exercise training.

“If the soreness then, be caused by the same conditions which produce fatigue, namely the presence of diffusible waste products of activity we should expect to find, as we do find, that it takes the course described above, passing away within few hours of the work itself” [1]

In 1900 Theodore Hugh first described that exercise induced muscle injury is not a fatigue phenomenon but the consequence of mechanical overload followed by structural and functional muscular changes [1].

Cycles of repetitive eccentric and concentric contractions represent a fundamental source of mechanical stress for active skeletal muscle and vulnerability of skeletal muscle fibers appears to be particularly evident in unaccustomed individuals. In fact conditioning of the muscle through prior similar activity may minimize damage appearance (so called “repeated bout effect”) [2]. Overall the process appears as a fundamental step for the arising of exercise-induced plastic response as it is followed by muscle remodeling and adaptation [3]. However muscle damage may delay muscle recovery from exercise and performance thus reducing the athletes compliance to exercise programmes.

The direct consequence of mechanical stress on active skeletal muscle fibers is the appearance of soreness (delayed onset muscle soreness, DOMS), stiffness and reduced force production. This is particularly the case as a consequence of strenuous physical work involving heavy resistance exercise including eccentric (i.e. lengthening) actions [4-6]. In these conditions force loss appears immediately post-exercise whereas soreness becomes evident within 24-48 hours after and, as the force impairment, may last several days depending of the extent of damage [7]. At the microscopic and submicroscopic level fibers damage, which preferentially involves fast twitch fibers [8], is already evident within minutes from the mechanical insult [9] displays throughout individual fibers (i.e. focal injury), and includes plasma membrane disruption accompanied by the loss of muscle proteins in the serum (i.e. creatine kinase (CK), myoglobin, lactate dehydrogenase (LDH), aldolase, troponin), myofibrillar disorders as streaming and broadening of the Z-lines, loss of sarcomeres register, the appearance of regions of overextended sarcomeres, regional disorganization of the myofilaments, subsarcolemmal lipofuscin granules accumulation, alterations of the proteoglycan components, increased interstitial space, and capillary damage [10-15]. Interestingly dramatic changes in the organization of the membrane systems involved in excitation-contraction coupling have been also found following eccentric contractions [16]. The most commonly identified alterations include disorders of the T tubule, changes in the direction and spatial orientation of the triads, and the appearance of caveolar clusters, pentads and heptads (close apposition of two or three T tubule elements with three or four elements with three or four elements of terminal cisternae of sarcoplasmic reticulum).

Apart mechanical stress, other mechanisms may contribute to muscle damage. In particular a metabolic impairment has been proposed as a result of ischemia or hypoxia during prolonged and intense resistance exercise. This insult leads to changes in ion concentration, accumulation of metabolic wastes, and adenosine triphosphate (ATP) deficiency which contribute to soreness and impaired function.

Importantly the early mechanical and metabolic mechanisms can promote biochemical changes within the affected area, leading to the generation of a secondary muscle damage including disruption of intracellular calcium homeostasis, local accumulation of inflammatory cytokines such as tumor necrosis factor- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6, and IL-1 receptor antagonist (IL-1ra) [17], *de facto* mimicking the sequential release of cytokines after trauma [18], and reactive oxygen species (ROS) that may further degrade muscle proteins and increase the local expression of cytokines [19, 20]. This condition may contribute to persistence of signs and symptoms of injury.

Although it is widely accepted that high intensity eccentric exercise is the fundamental exercise paradigm resulting in muscle damage and subsequent adaptation, structural and functional damage may also arise following long lasting endurance exercise paradigms as demonstrated by the appearance of ultrastructural alterations, as fibers necrosis, sarcolemmal disruption, Z discs streaming, contracture knots, and inflammatory infiltration in endurance athletes even before a race [21]. Anyhow even though the extent and location of damage may greatly vary according to the exercise paradigm and the previous conditioning of the musculoskeletal system, the extent of damage observed with low intensity and long duration endurance exercise is often less pronounced than with higher intensities. This is the main reason of why most of works finalized to the identification of the physiological mechanisms that regulate the response to exercise-induced stress in skeletal muscle and the possible countermeasures, including the approach based on nutrient supplementation, have been focused on strength training exercise.

## 2. Nutritional intervention to minimize exercise-induced muscle injury

For decades, dietary supplementation has been proposed in various physiological or pathological conditions. Based on the recent progress in our understanding of the cell signaling and *in vivo* metabolism of nutrients and on accumulating experimental results, the concept that dietary supplementation might have effects in prevention or treatment of several disorders is experiencing a new revival. To date several investigations have been focused on accumulating experimental evidence aiming to extend the use of specific nutritional supplements in the prevention and/or treatment of exercise induced muscle injuries. Anyhow available outcomes on potentially efficacious supplements are mixed and often conflicting and confident conclusions cannot be drawn. Several variables may concur to contradictory results including the wide number of supplements to be considered, their combined use, their dose and timing of administration. Furthermore the choice of the proper indexes to be analyzed is certainly a major bias for several published studies on the topic as a misinterpretation of results may follow the analysis of indirect instead of direct signs of muscle injury. Thus, although many nutrients are potentially able to impact on the mechanisms underlying the appearance of muscle damage following exercise, the final efficacy and safety of their supplementation deserves future rigorous investigation.

The present chapter discusses the potential role of antioxidants, creatine, carnitine and branched chain amino acids on exercise induced muscle damage.

### 3. Antioxidant/vitamines

Nutritional antioxidants are non enzymatic compounds including the lipid-soluble vitamin E,  $\beta$ -carotene, co-enzyme Q10 (CoQ), and the water-soluble vitamin C, glutathione, and uric acid. These antioxidants either scavenge ROS into less reactive molecules or prevent their transformation into more highly reactive forms, having intracellular and extracellular sites of action [22].

During exercise and exercise-induced damage whole body oxygen consumption increases up to 20 fold and ROS are generated in excess [23]. The primary sources of ROS are endogenous sites within the skeletal muscle, whereas the secondary sites of production are exogenous [24]. Within the muscle the main font of ROS is reputedly through electron leakage in the mitochondria during mitochondrial phosphorylation and via xanthine oxidase metabolism in the capillary endothelium [25, 26] whereas the main secondary source of ROS is generated during the inflammation mainly by neutrophils.

Considering that increased ROS production could challenge the natural antioxidant defense system and that ROS play a major role in the initiation and progression of exercise-induced skeletal muscle injury [27, 28], it has been hypothesized that antioxidants supplementation may minimize its extent and this topic has been faced by a plethora of studies. However, to date, strong evidence to support significant reductions in structural or functional impairment due to antioxidants is missing. Inconsistencies of findings may relate to ununiformities in the experimental designs in terms of type, dose, time of administration and chosen indexes to evaluate and quantify muscle injury. In fact the majority of investigations have been focused on the effects of vitamin C and E and looked at changes in plasma concentrations in CK and LDH and oxidative stress markers. Much less studies have analyzed direct indexes of muscle damage as loss in muscle strength, soreness and structural/ultrastructural changes of the fibers [26, 29]. Indeed a not univocal strategy in the timing of supplementation (pre exercise, during exercise, post exercise) has been adopted demonstrating *de facto* a lack of a univocal and definite and generally accepted mechanism underlying the correlation between exercise, muscle damage, and antioxidant activity. As a matter of facts several studies have examined the effects of antioxidants on indices of ROS-induced muscle damage in exercise and suggested that antioxidant supplementation may exert some protection particularly in relation to bouts of resistance exercise in untrained or physically active individuals [30-35] as demonstrated by a reduced inflammation [35-39], force loss [30, 40, 41], and fatigue appearance [42, 43] and no evidence for any beneficial effect on performance [44]. On the contrary no significant effects of antioxidant supplements have been found by other authors on indices of inflammation [45-49], cell damage [45, 48, 50-53], oxidative stress [53], and muscle soreness [54-57]. The lack of effects appears to be particularly evident in highly trained individuals whose adaptation to increased exposure to oxidation is normally able to promote a secondary increase of the endogenous antioxidant defenses that reduce the risk of oxidative damage [58, 59] Therefore even following extreme exercise paradigms, unlike short periods of modest exercise [60], indications of oxidative damage may lack in well trained athletes [61]. Importantly in these conditions exposure to antioxidants may hinder the beneficial cell adaptations to exercise



thereby promoting muscle damage instead of recovering from it [55, 62-64]. In fact there are concerns about possible adverse effects of megadose supplementation, as several of these nutrients have been shown to increase markers of exercise-induced oxidative stress thus serving as prooxidants instead of antioxidant nutrients. For example after intense exercise, supplementation with vitamin C, vitamin E, N-acetylcysteine and coenzyme Q10 was associated with oxidative stress, increased serum CK and, in some cases, reduced performance [63-67]. In another study prolonged (2 months) vitamin E supplementation increased lipid peroxidation and inflammation [68] whilst no effects on resting levels of oxidative stress were observed by others following vitamin C and E supplementation in ultraendurance athletes [69].

*Vitamin C.* To date there is no evidence to support the hypothesis that acute and prolonged supplementation with ascorbic acid before and/or after exercise may prevent/attenuate exercise induced muscle damage.

Although a long lasting supplementation with vitamin C before exercise bouts has met with conflicting results, some of them reporting a beneficial effect on lipid peroxidation and inflammation [70] probably due to an increase of the baseline response of antioxidant enzymes [71], nowhere a clear effect on muscle damage has been reported. Similar results have been obtained following acute supplementation prior to exercise. In particular an early study by Ashton and colleagues [72] demonstrated a protective effect of an acute dose of ascorbic acid against ROS production following exhaustive exercise. In this study no indices of muscle damage were measured. Later the effects of an identical dose of ascorbic acid 2 hours before 90 minutes of intermittent shuttle running were investigated by others [52]. Supplementation did not affect the increases in serum CK, serum aspartate aminotransferase, and delayed onset muscle soreness [52]. Importantly the same negative results were obtained when supplementation was performed after training. In particular vitamin C supplementation for 3 days after an intermittent shuttle running showed no effects on indexes of muscle damage, lipid peroxidation and inflammatory response [56]. In another study combination of vitamin C with n-acetylcysteine for 7 days after an eccentric bout of exercise exerted a prooxidant effect. Furthermore equivocal results emerged when supplementation was given before and after exercise bouts, as the smoothening effects on DOMS observed by some authors [34, 73] have been not confirmed by others [54, 55].

Overall, although conflicting results on the topic may result from ununiformities of supplementation strategies and inconsistencies in the experimental procedures adopted (for example the lack of crossover design or the missing measures of direct indexes of muscle damage), there is limited evidence of a protective effect of vitamin C on exercise induced muscle damage.

*Vitamin E.* Vitamin E, the most important lipid-soluble antioxidant vitamin, is known to stop the progression of the lipid peroxidation chain reaction and is an important scavenger of the superoxide, hydroxyl and lipid peroxy radicals [74]. Vitamin E can be recycled from its radical form by vitamin C and less efficiently by other antioxidants (glutathione, CoQ, cysteine and  $\alpha$ -lipoic acid). Importantly, this vitamin may also act as a prooxidant in the absence of these antioxidants [75]. Most of studies investigating the effects of vitamin E on exercise induced muscle damage have utilized a preexercise supplementation strategy starting from the assumption that vitamin E, contrarily to vitamin C, being lipid-soluble can be stored in tissues.

Anyhow direct indexes of muscle damage were not adequately measured in most cases and available results, albeit suggesting a minimal, unless absent [45], protection of vitamin E supplementation on oxidation, membrane damage [76, 77], and inflammation [76], do not provide enough evidence for a protective effect of vitamin E on exercise induced muscle damage.

In conclusion, there is little evidence to support the suggestion that supplementation with antioxidant nutrients can improve exercise performance, but there is a growing body of evidence to suggest that supplementation may reduce the extent of exercise induced oxidative damage. If this is indeed the case, it may be that the athlete undertaking a strenuous training programme may benefit in the long term by being able to sustain a higher training load (less fatigue). There is also evidence, however, that prolonged exposure to training increases the effectiveness of the endogenous antioxidant mechanisms, and it may be that supplementation is unnecessary or prooxidant and thus potentially unsafe.

#### 4. Carnitine

Carnitine is the required carrier of fatty acids from the cytoplasm into mitochondria, where they undergo  $\beta$ -oxidation [78, 79]. In the cytoplasm carnitine combines with fatty acyl-coenzyme A (acyl-CoA) thus allowing that fatty acids may enter the mitochondrion. The first step of this process is catalysed by carnitine palmitoyl transferase 1 (CPT1) and the transmembrane transport is facilitated by acylcarnitine transferase. Within the mitochondrion free carnitine is regenerated by the action of carnitine palmitoyl transferase 2 (CPT2) and the released fatty acyl-CoAs enter the  $\beta$ -oxidation pathway. Within the mitochondrion, carnitine also regulates the acetyl-CoA concentration and the concentration of free CoA. Considering that free CoA is involved in the pyruvate dehydrogenase reaction and in the process of  $\beta$ -oxidation it contributes to the coordinated integration of fat and carbohydrate metabolism. In fact when glucose oxidation increases, acetyl groups can be translocated from acyl-CoA within the mitochondrial matrix to the cytoplasm. The accumulation of cytosolic acetylcarnitine may result in a limitation of CPT-1 activity because of the decrease in availability of free carnitine. Consequently, there is fatty acid oxidation, since skeletal muscle predominantly expresses an isoform of CPT-1 with low affinity for L-carnitine [80].

In humans, 75% of carnitine is obtained from the diet. The primary dietary sources of carnitine are red meat and dairy products [81]. Dietary carnitine is absorbed from the intestinal lumen across the mucosal membrane by both passive and active transport mechanisms. Carnitine is also synthesized in the liver and in the kidneys (not in skeletal and cardiac muscle) [82] from the essential amino acids, lysine and methionine [83, 84] having ascorbic acid, ferrous iron, pyroxidine, and niacin as necessary cofactors [85]. More than 95% of the body's total carnitine store is within skeletal muscle tissue [86], and decreased plasma carnitine level has been related to low tissue concentrations [79, 87].

Considering the key roles of carnitine for normal skeletal muscle bioenergetics (long-chain fatty acid oxidation; removal of acyl groups from the mitochondria; detoxification), its

availability may be the limiting factor for fatty acid oxidation and/or the removal of acyl-CoAs also during exercise [82]. Based on such considerations it has been proposed that carnitine consumption may improve exercise performance and/or recovery from exercise. Consistently the large majority of studies observed a beneficial effect of L-carnitine supplementation on maximum oxygen uptake or respiratory quotient in healthy athletes [88] whereas only a minority of studies failed to observe such effects [88]. In particular, several scientific reports highlight that carnitine supplement could be an ergogenic aid for endurance exercise [89, 90] as in presence of concomitant low carnitine concentration in skeletal muscle limiting carnitine acyltransferases to operate at a high rate, the oral ingestion of carnitine would result in an increase of the total carnitine concentration. This effect may be followed by increased rate of oxidation of intramuscular fatty acids and triacylglycerols during exercise thus reducing muscle glycogen breakdown and postponing fatigue appearance [88]. On the other hand a decrease of free carnitine concentration to very low levels is expected in skeletal muscles subjected to high-intensity training because the compound tends to react with acetyl-CoA. This decrease has been suggested as one of the mechanisms for the reduction of plasma fatty acid and intramuscular triacylglycerol oxidation during high-intensity exercise [91]. Accordingly most studies showed improved maximum oxygen consumption, reduced lactate accumulation, and increased high-intensity exercise performance in professional and nonprofessional athletes, especially when L-carnitine was supplemented for longer periods and at higher doses [92-95]. However, some investigations failed to show any effect of carnitine supplementation following on high-intensity training programs [96-101].

Recently a discrete bulk of research has provided the evidence to support the theoretical potential for the use of L-carnitine supplementation in exercise recovery. These studies demonstrated that supplemental carnitine is effective in attenuating tissue damage as directly assessed via magnetic resonance imaging, muscle soreness, and postexercise markers of metabolic stress following eccentric exercise training [102] or intense resistance exercise [103-105] thus leading to a quicker recovery (2 to 3 g/day of elemental carnitine being supplied by L-carnitine L-tartrate, LCLT). In particular Volek and colleagues [103] analyzed the effects of L-carnitine (2g/d for 3 wk before exercise and during 4d recovery) on markers of muscle damage in trained adult man following 5 sets of 15-20 repetitions of squats at 50% of 1-RM. Treated subjects experienced reduced muscle damage and decreased circulating CK compared to placebo. Similar results, recently obtained by the same authors, have clearly shown that LCLT is also effective in promoting recovery of tissue damage arising from the same protocol of high-repetition squat exercise in elderly individuals [106].

Overall the observed benefits of L-carnitine supplementation in preventing exercise-induced muscle injury have mostly been attributed to its potential as antioxidant. Increased generation of ROS is considered as a major cause of disruption/damage to the sarcolemma leading to leakage of cytosolic proteins into the circulation (CK, myoglobin, LDH). Furthermore ROS generated beyond physiological limits are found to reduce muscle force production by altering calcium ion sensitivity in muscle and thus contributing to muscle fatigue [107] [108, 109]. L-carnitine supplementation has been related to reduced postexercise CK [102, 103] and myoglobin [103, 105] concentrations suggesting that reduced oxidative stress may play a role in a



quicker muscle recovery from strenuous exercise following supplementation. As a matter of fact early studies by Brass et al. (1993) [110] demonstrated that L-carnitine delays hypoxia-induced fatigue in electrically stimulated rat skeletal muscle *in vitro* through its key stimulatory role in muscle bioenergetics and antioxidant activity. Further evidence demonstrated that L-carnitine exhibits effective superoxide anion radical and hydrogen peroxide scavenging, total reducing power and metal chelating activities *in vitro* [111]. *In vivo*, in presence of high glycolytic rates as during strenuous resistance exercise, the stimulated formation of ATP and AMP from molecules of ADP results in the oxidation of AMP to hypoxanthine which is considered a marker of metabolic stress [112]. This oxidation reaction is mediated by xanthine oxidase. Accumulation of xanthine oxidase in spite of xanthine dehydrogenase is the consequence of the activation of calcium-dependent proteases, which cleave a portion of xanthine dehydrogenase and convert it into xanthine oxidase. This process is a direct consequence of raised intracellular calcium by inhibition of calcium ATPase pumps induced by insufficient supply of ATP. This response appears to be attenuated by L-carnitine supplementation which reduces intracellular hypoxanthine and xanthine oxidase following resistance exercise bouts [103, 106]. Indeed inhibition of xanthine oxidase with allopurinol during exercise has been shown to result in significantly less generation of ROS, reduced tissue damage after exhaustive exercise [112], and less accumulation of cytosolic enzymes CK and LDH [113, 114]. Furthermore a direct consequence of high-intensity training is hypoxia. Exercise under hypoxic conditions stimulates muscle glucose transport, increases the concentration of ammonia in blood, and lowers the concentration of free carnitine [115, 116]. It has been found that carnitine supplementation during exercise under hypoxic conditions may also prevent ammonia toxicity mainly through reduction of ROS production.

In summary, L-carnitine supplementation can beneficially affect postexercise markers of metabolic stress, muscle disruption, and muscle soreness in young and old healthy men and women. The attenuation of the side effects of high-intensity training mainly relate to its antioxidant potential and its capability to reduce the magnitude of exercise-induced hypoxia. Further research is needed to conclusively elucidate the mechanisms underlying its protective effects and whether these responses may also arise in exercised individuals affected by disorders of different origin as neuromuscular diseases.

## 5. Creatine

A large number of surveys indicate that creatine (n [aminoiminomethyl]-N-methylglycine) is one of the most widely used nutritional supplements [117-122]. Prevalence studies indicate that the use of creatine is particularly common in athletes and soldiers. Among the athletes population, powerlifters, boxers, weightlifters, and track/field athletes report the higher creatine consumption with prevalence ranging between 45 and 75% [122]. The major determinant of such a widespread consumption by resistance athletes mainly resides in the known ergogenic aid of creatine when supplementation is associated with repeated bouts of high intensity exercise. This combination leads to increased lean body mass (with no effect on fat mass), muscle strength and performance and accelerated post-exercise recovery [123].

Interestingly more pronounced effects of creatine supplementation have been found in strength trained older adults compared to the young adults [124] and in untrained compared to trained individuals whereas similar changes in muscle creatine content and exercise performance have been found between men and women [125, 126]. Besides no ergogenic effect of creatine has been found in a variety of endurance exercise paradigms [127-129].

Several mechanisms could explain the effects of creatine supplementation on muscle mass, strength and performance when supplementation is combined with strength training.

The hypertrophic response has been attributed to increased myosin heavy chain protein expression [130], changes in the expression of myogenic regulatory factors (MRF4 and myogenin) [131, 132], increased mitotic activity of satellite cells and swelling-induced protein synthesis [133-137] followed by net protein deposition. The most popular mechanism to explain the efficacy of creatine on muscle performance refers to a better match between ATP supply and fibers demands during physical exercise due to the enhancement of the resting high energy phosphate levels (total creatine, phosphocreatine, creatine and ATP) observed following supplementation. This change allows users to maintain a greater work intensity for longer durations of time (increased total training volume). In particular, the intracellular concentration of phosphocreatine is known to play a major role during the bioenergetic system mostly active during exercise at high intensities and short durations. Overall the dosing regimen that has been found to significantly increase the intracellular phosphocreatine is a loading phase of approximately 20 g/day for 5-7 days followed by a maintenance phase of 5 g/day for a period of several weeks [138, 139].

The known effects of creatine upon muscle cell function, structure and protein metabolism may represent the rationale for its potential use to prevent or treat muscle cell injuries. Nevertheless, although solid studies have examined the ergogenic potential of creatine, the current literature is very preliminary in relation to examining the effects of creatine supplementation in reducing the severity of exercise-induced muscle damage and/or promote recovery following strength training and endurance paradigms [140].

Considering that high-force eccentric exercise alters myofibre membrane structure and function [9, 141] leading to reductions in force, increased soreness, and impaired muscle function and that membrane stabilization due to decreased membrane fluidity is followed to increased intracellular concentration of phosphocreatine [142], the effects of creatine supplementation on markers of eccentric exercise damage have been assessed following resistance exercise sessions [143-146]. Initial studies conducted in rodents and humans agreed to demonstrate that creatine supplementation does not decrease muscle damage or enhance recovery after high intensity eccentric contractions. In particular Warren and colleagues [143] demonstrated that recovery of mouse anterior crural muscle strength after damage induced by 150 eccentric contractions was unaffected by creatine supplementation at 0.5 and 1% for two weeks. Following 3 minutes recovery, there was no effect of creatine supplementation on the isometric torque loss or on the torque loss at any eccentric or concentric angular velocity tested [143]. In 2001 Rowson and colleagues [146] evaluated the effects of short time creatine and dextrose supplementation (20 gr d-1 creatine and 28 gr d-1 for 5 days, a protocol previously shown to be effective in elevating muscle creatine and phosphocreatine levels [126]) before

performing 50 maximal eccentric contractions of the elbow flexors on blood markers of muscle damage (CK and LDH), maximal isometric force, range of motion, arm circumference (an index of swelling), and muscle soreness. Despite the initial hypothesis, results showed nearly identical loss of maximal isometric force and range of motion, development of soreness, increase of the biceps circumference and change in blood CK and LDH in supplemented and placebo groups of subjects thus suggesting that creatine supplementation lacked to display significant improvement of membrane stabilization at the conditions analyzed. In a second study by the same authors male participants were supplemented with creatine for 5 days prior to, and 5 days following a hypoxic resistance exercise test (5 sets of 15-20 repetitions at 50% of 1 repetition maximum). Similarly to the first study creatine failed to have positive effects on the same criterion measures of muscle damage following the resistance exercise challenge [145]. More recently differing results have been obtained following creatine and carbohydrate supplementation to untrained male subjects by the scheme 5 days prior to, and 14 days following a resistance exercise training session consisting of 4 sets of 10 eccentric repetitions at 120% of maximum concentric 1-RM on the leg press, leg extension and leg flexion machine. Creatine supplementation produced significantly greater isokinetic and isometric knee extension strength during recovery from exercise-induced muscle damage. Furthermore, plasma CK activity was lower after 48, 72, 96 hrs, and 7 days recovery in the supplemented group [144]. As discussed by Cook such diverse observations could be in part attributed to the duration of supplementation period and/or post-exercise supplementation. In particular in the first study by Rawson the subjects enrolled were supplemented only for 5 days prior to the exercise protocol; with no continuation of supplementation following the exercise bout [146]. As it has been suggested that the effect of creatine on protein synthesis and muscle regeneration may be enhanced during the recovery period post-injury [130, 147], the time schedule of creatine supplementation respect to the exercise bout may be considered a potential limiter of the muscular protection against exercise-induced damage. This hypothesis seems to be confirmed by the observed increase of satellite cell number and myonuclei concentration following creatine supplementation in human skeletal muscle [147]. Indeed it can be hypothesized that this effect may sum to the known training-induced increase in muscle regeneration.

Notwithstanding supplementation was continued for 5 days after the exercise bout, in the second study by Rawson and colleagues no beneficial effects of creatine on criterion measures of muscle damage were observed [145]. Although it cannot be excluded that the resistance exercise paradigm used by Rawson, designed to be hypoxic in nature, may not have elicited enough muscle damage to unmask the anabolic effects of creatine supplementation, to date available conflicting data from a limited number of experimental works on the topic do not allow to safely draw conclusions on the beneficial effects of oral creatine supplementation on skeletal muscle damage and recovery following eccentric exercise challenge and new, more standardized, experimental works would help unravel this question in the next future.

Based on the fact that cell injury in running depends on cell volume integrity and that creatine potentially stabilizes the cell volume through an increase in cell water content, glycogen stores and/or myofibrillar content [135-137, 148], the effect of oral creatine supplementation has also been examined on markers of muscle damage, i.e. inflammatory and muscle soreness markers,

following prolonged running exercise (30Km run) [149]. Marathon runners were supplemented for 5 days (20 g/day) prior to a 30km race. Blood samples were collected pre-race, and 24 hours following the end and CK, LDH, prostaglandin E2 (PGE2) and TNFalpha (TNF- $\alpha$ ) were measured.

As expected, prolonged running provoked an increase in concentrations of all plasma markers tested, indicating the appearance of cell injury associated with an inflammatory response [6, 150]. Creatine supplementation was effective in significantly attenuate the observed increase in all muscle soreness markers analyzed, unlike CK, thus pointing this nutritional intervention as an effective strategy in maintaining muscle integrity during and after intense and prolonged endurance exercise. In fact the lack of effect upon plasma CK concentration might not reflect the overall positive effect of creatine on muscle damage as a strong variability of this parameter among athletes, its dependence from the training status and the weak correlation with changes in other markers of muscle damage [151-153] lessen its significance in comparison with other markers of cellular death and lysis as LDH [154]. Similar effects on plasma pro inflammatory markers (Interleukin (IL) 1 beta and IL-6, TNF- $\alpha$ , and Interferon alpha (INF alpha) and PGE2) [155] and on plasma markers of cellular integrity (CK, LDH aldolase (ALD), glutamic oxaloacetic, acid transaminase (GOT), glutamic pyruvic acid transaminase (GPT), and C-reactive protein (CRP) [156] have been obtained in double blind trials following creatine supplementation (20gr day<sup>-1</sup>) 5 days before a half-ironman and after ironman triathlon competition respectively.

These results confirm the hypothesis that creatine may have a protective effect against membrane cell disruption following prolonged and intense muscle contractions [156]. Indeed as skeletal muscle damage during an ironman competition mostly result from eccentric contractions, mainly related to the marathon segment of the race [157], it can be argued that creatine may be effective in preventing eccentric induced muscle injury. In fact exhaustive exercises involving eccentric contractions, as in triathlon competition, lead to more pronounced muscle damage than strenuous exercises involving concentric contractions [158]. Nevertheless other mechanisms but eccentric damage may contribute to muscle damage during triathlon competition including excessive metabolic workload, muscle fatigue, depletion of intramuscular glycogen, and oxidative stress which are generally implicated in prolonged exercise-induced muscle fiber disruption [157, 159, 160]. The observed reduction in plasma activities of GOT and GPT (markers of liver injury) observed in triathletes after an ironman competition may suggest that creatine supplementation can enhance the metabolic efficiency of skeletal muscle preventing the metabolic workload on the liver which has a critical role on the contractile activity-induced skeletal muscle injury. Indeed when eccentric contraction is avoided as in electrically stimulated gastrocnemius muscle of the rat, creatine supplementation has been found to delay the fatigue appearance, preserve the force development, and prevent the rise of LDH and CK plasma activities and muscle vascular permeability evaluated with Evans blu staining [156]. Furthermore, although it cannot be excluded that in endurance settings the benefits of supplementation in preventing muscle damage may relate to the antioxidant potential that has been attributed to creatine in various oxidative stress-associated diseases, few studies have been published on the relationship between supplement-



tation and oxidative stress and controversial and not conclusive results are currently available [161-163]. In particular creatine supplementation associated with resistance training or exhaustive exercise training has been associated either with reduced oxidative stress [162, 164], increased free radical generation and related consumption of antioxidant reserves [161] or no change of lipid peroxidation, resistance of low density lipoprotein to oxidative stress or plasma concentrations of non-enzymatic antioxidants [163]. Taken together these observations show that creatine supplementation before strenuous endurance exercise reduces the increase of markers of cell death/lysis and muscle soreness suggesting a positive effect of the supplementation strategy in maintaining muscle integrity after intense prolonged exercise. The mechanisms underlying such a protective effect are only partially known.

## 6. Essential amino acids

Considering that mechanical stimuli may induce skeletal muscle damage as a consequence of overload and/or eccentric actions causing cytoskeleton and subcellular disruption on muscle fibers, it appears that nutritional interventions finalized to maximize protein synthesis (MPS) or minimize protein catabolism (MPC) would be helpful in preventing and/or treating exercise induced muscle injuries.

As known MPS includes the complex process of mRNA translation which develops in three consecutive steps i.e. initiation, in which the initiator methionyl-tRNA and mRNA bind to 40S ribosomal subunit, elongation, by which tRNA-bound amino acids are incorporated into growing polypeptide chains according to the mRNA template, and termination, where the completed protein is released from the ribosome [165].

The first two steps of mRNA translation are highly regulated at two different levels: the binding of methionyl-tRNA to 40S ribosomal subunit to form 43S preinitiation complex, and recognition, unwinding, and binding of mRNA to the 43S, catalyzed by a multi-subunit complex of eukaryotic factors (eIFs), referred to as eIF4F. The mammalian target of rapamycin (mTOR) kinase which is now recognized as a key regulator of cell growth and a pivotal sensor of nutritional status, is a key regulator of MPS. In cells, mTOR forms two distinct complexes, mTORC1 and mTORC2, depending on the binding partners. When bound to raptor (regulatory associated protein of mTOR) mTOR forms mTORC1, which mediates the effects sensitive to rapamycin [166]. mTOR-mediated regulates protein synthesis is based on Activation of eIF4E binding protein-1 (4E-BP1) releases the inhibition on the eukaryotic factors complex eIF4F, which is responsible for the interaction with 40s ribosomal subunit and translation initiation [167]. In fact when 4E-BP1 is in its hypophosphorylated state it blocks the ability of eIF4E to bind to eIF4G and forms an inactive 4E-BP1-eIF4E complex. This interaction precludes mRNA to bind to the ribosome. mTORC1 is also responsible for the activation of downstream S6K1. S6K1 is a kinase which requires phosphorylation at two sites and its activation is necessary for muscle fibres to achieve normal size, since S6K1 knockout cells are smaller than control cells [168]. Following phosphorylation at Thr389 by mTORC1, S6K1 regulates the activity of eukaryotic elongation factor 2 kinase (eEF2k) [169].



Studies in animals models and humans showed that essential amino acids (EAA) [170, 171] unlike non EAA [170], are fundamental regulators of MPS and mitochondrial biogenesis [172]. It has been shown that hyperaminoacidemia stimulates amino acid transport and net MPS, unlike carbohydrate administration both in the young [173] and in the elderly [174]. The effects on protein synthesis arise independently of changes in anabolic hormone concentration [175, 176], although insulin is required for the effects of EAA on translation [177]. Among EAA, Branched chain amino acids (BCAA: leucine, isoleucine and valine) play a very important role as nutrient signals that regulates MPS through the stimulation of insulin-independent and rapamycin-sensitive pathways [178, 179]. In particular available data suggest that at least part of the postprandial translational activation is to be attributed to BCAAs through activation of mTOR and downstream signals (eIF4G, S6K1 and 4E-BP1). Although mTOR is the key integrator of the anabolic response to BCAA, mTOR itself may not be the direct target of EAA. It has been shown that inhibition by the upstream TSC1/2 complex represents the mechanism through which leucine and insulin upregulate mTOR and downstream targets.

The scenario arising from available studies indicates that the physiological anabolic response to BCAA may help counteracting the metabolic unbalance induced by exercise and in particular resistance training which has been linked to concurrent increase of MPS and MPC [180, 181] and negative changes in circulating free amino acids [182]. In these conditions the exercise-triggered hypercatabolism may be counteracted by amino acids supplementation which in turn has been related with net protein synthesis when combined with bouts of resistance exercise [173, 183, 184] and prevented BCAA exercise-induced oxidation [182].

Recent studies suggest that BCAA supplementation, by promoting MPS, may improve the repair of muscle damage induced by resistance exercise.

In particular Nosaka et al. [185] showed that an amino acid supplement containing around 60% BCAA was effective in reducing muscle damage and soreness when consumed immediately before (30 min) and during the four days of recovery following a damaging bout of lengthening contractions of the elbow flexors. Later Jackman and coworkers reported the effects of BCAA supplementation during recovery from intense eccentric exercise consisting in 12 x 10 repetitions of unilateral eccentric knee extension in male untrained subjects. A decrease in flexed muscle soreness was observed in supplemented compared with placebo group at 48 h and 72 h post exercise whereas the degree of force loss and the fluctuation of blood markers of muscle damage appeared unchanged between groups [186]. Similar results were obtained in female untrained young subjects by Shimomura et al. [187] examining the effects of BCAA supplementation on squat-exercise-induced DOMS. In this report the participants ingested either BCAA (isoleucine:leucine:valine = 1:2.3:1.2) or dextrin at 100 mg/kg body weight just before the squat exercise consisting of 7 sets of 20 squats/set with 3-min intervals between sets. The peak of DOMS was reached two or three days post exercise but the level of soreness was significantly lower in the BCAA trial than in the placebo. Interestingly three day post exercise the force decrease observed in the placebo appeared to be prevented by BCAA supplementation. Accordingly plasma myoglobin and elastase (index of neutrophil activation) appeared to be increased by exercise in the placebo but not in the BCAA group [187].

Interestingly the beneficial effects of BCAA mixtures supplementation has been reported also following moderate resistance training and endurance training both in rodents [188] and humans [189-191]. In these conditions the effect of supplementation seems to mainly reflected on a reduced rate of perceived exertion (RPE) [189] and reduced proteolysis as demonstrated by reduced phenylalanine release from the muscle [192], whereas no beneficial effects have been found in terms of changes in exercise performance [189]. In particular in the study by Greer and coworkers nine untrained male subjects were supplemented with a BCAA enriched beverage, an isocaloric, carbohydrate (CHO) beverage or a noncaloric placebo beverage. The subjects performed three 90-minute cycling bouts at 55% VO<sub>2</sub> peak followed by 15-minute time trials and ingested a total of 200 kcal via the CHO or BCAA beverage before and at 60 minutes of exercise or the placebo beverage on the same time course. A greater distance was traveled during the CHO trial than the BCAA and placebo trial. On the contrary the RPE was reduced during the BCAA trial as compared with the placebo trial. This study clearly demonstrated that CHO supplementation improved performance compared with BCAA and PLAC beverage. Thus BCAA supplementation did not influence aerobic performance but attenuated RPE [189]. Accordingly BCAA supplementation (0.8% BCAA in a 3.5% carbohydrate solution; 2,500 mL/day for four days) effectively reduced the muscle soreness and fatigue sensation when supplementation was carried out during an intensive endurance training programme in male and female, and the perceived changes could be attributed to the attenuation of muscle damage as demonstrated by decreased LDH, CK and granulocyte elastase levels, and inflammation [190, 191].

Importantly a minority of works contradict the general findings from other research on the benefits of BCAA on resistance exercise muscle damage. In particular conflicting results have been reported by Stock et al. [193] showing that in a mixed sex group of trained participants there were no differences in damage indices of resistance exercise (6 sets of squats to fatigue using 75% of the 1 repetition maximum) between a carbohydrate versus a carbohydrate/leucine supplement. The subjects enrolled consumed the carbohydrate beverage 30 minutes before and immediately after exercise with or without the addition of 22.5 mg kg<sup>-1</sup> of leucine. Results showed that the addition of leucine did not significantly decrease CK and LDH activity or DOMS evaluated at different time points following exercise thus suggesting that adding leucine to carbohydrate beverages did not affect acute muscle recovery from exercise. Considering that in the study by Stock and coworkers the amino acid supplement consisted of leucine alone (and not of a mixture of BCAA), one can speculate that a methodological bias may account for the observed different outcome of this study compared to others.

In conclusion the overall effect of resistance exercise on circulating BCAA suggests that exercise induced muscle damage is followed by an increase of skeletal muscle BCAA uptake from the serum being used as energy source and/or participate in translation initiation signaling pathway involved in muscle remodeling. Functionally this appears to have some consequence in muscle pain. A similar effect on the rate of perceived exertion has been found following BCAA supplementation before and during endurance exercise, when muscle remodeling is reasonably much less than in resistance exercise. The mechanisms beyond the

protective effects of BCAA supplementation on muscle damage deserve further investigations, to be mostly oriented on unraveling the effects of supplements on inflammation.

## 7. Conclusions

The skeletal muscle is placed under considerable stress during high repetitive eccentric, or lengthening, contractions.

Several studies have used a variety of nutritional supplementation strategies including macronutrients and micronutrients, with variations in dosage, timing and duration of supplementation, finalized to minimize exercise induced muscle injury. Although there is proper rationale and some evidence showing the efficacy of certain supplements such as creatine and essential amino acids, there is little evidence to support a role for others including the antioxidants. Indeed, antioxidant supplementation may interfere with the cellular signaling paths thereby unfavorably affecting muscle function, performance, and recovery from injury.

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