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Metabolic and hormonal basis of fatigue during exercise

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ABSTRACT

Exercise-induced reduction in maximal force production or the inability to continue activity with enough force is defined as fatigue. Although the etiology of fatigue is complex, but it can be divided into two distinct parts: Central and peripheral which are not separated from each other and have close relationship with each other. Different activities cause fatigue and main challenge is identifying the different mechanisms which are involved in various conditions. Seemingly the traditional justification of the intra cellular accumulation of lactic and hydrogen ions, which cause to dysfunction of contractile protein in mammals, particularly humans, has little significance. In one hand most of the studies about fatigue has been done on isolated animal fiber and in another hand the main issue is putting the dispersed information from different studies together in order to understand the fatigue mechanism in human, particularly athletes. Topics which will be discussed in this study would complete our understanding of the metabolic and hormonal fatigue.

INTRODUCTION

A muscle used in high-intensity, experiences progressive decrease in performance that recovers after a short period of rest. This reversible phenomenon is called fatigue [1]. Statistically, neuromuscular fatigue can be defined as any reduction in force generation capacity. During a static maximum contraction, force will be decreased continuously and fatigue will be observed from the beginning of the exercise. In contrast, target force is maintained for a long time under maximal contractions. In this condition, the fatigue is defined as the inability in maintaining the force, even if the capacity to produce maximum force before and during contraction is impaired. Usually neuromuscular fatigue is defined as a reduction in the maximum force generating capacity [2]. Vollestad [3]defined neuromuscular fatigue as any reduction in maximum capacity of force generation or output because of the exercise. This definition allows us to define fatigue in different sports and different intensities. In addition, we should make a contrast between the muscular weakness as a chronic disorder in generating the force or output, and acute effect of neuromuscular fatigue. Therefore, it seems that neuromuscular fatigue develops differently, depending on the muscular activity form[4].

From long time ago, fatigue has been considered by human ancestors, battle warriors and athletic championships to preventor at least delay it by doing some devices. Even now, preventing fatigue and performance degradation is the concern of professional athletes who seek colorful medals and championship platforms. This requires knowledge of the causes and mechanisms underlying fatigue. In this paper, the metabolic and hormonal basics of the fatigue will be studied in order to obtain a thorough perception of this point of view.

Sporting activities are accompanied by the changes in metabolite levels in which the magnitude of the changes depends on the type of activity. For example, activities with working load more than the critical power results to

rapid decrease in PCr, while the concentration of P_i and PH increases steeply[5]. In mice, which had to swim 30, 60 and 120 min, beta butyric acid, acetate, lactate, and lipids significantly increased and glucose, choline, phosphoryl choline, alanine and phosphatidylcholine decreased. These changes were dependent to the time[6]. Interestingly, metabolic changes, and thus a reduction in force in the fast-twitch fibers are more than the slow-twitch fibers [1]. It seems increase in ATP hydrolysis products (ADP, P_i and H^+) levels, has a negative effect on contraction parameters, so probably during the intense sport activity these products get involved in the fatigue process [7]. In another hand, afferents of pain/metabolic receptors (group III / IV) which are originated from the active muscles are stimulated by side products of Intramuscular metabolism [8], and in parallel with the accumulation of metabolites, their input to CNS is increased. This Central projection causes to increase in blood flow (pressure reflex induced by vasoconstriction which is induced by exercise),ventilation[9]reflexively, gradually decreasing central motor output / voluntary muscle activation and increases central fatigue [2].Therefore, the effect of every metabolic product on energy production and fatigue development is discussed in the next part.

A) Inorganic phosphate

During exercise, concentration of P_i increases as a result of cytosolic PCr depletion. Experiments with skinned fiber show that calcium release from sarcoplasmic reticulum (SR) is decreased by P_i increase[7]. Intact(complete) fibers test shows that reduction of calcium release is associated with a similar reduction in the total amount of calcium that may be released by caffeine [7]and lessen the reduction of calcium release with preventing P_i increase (CK Inhibition)[7]. These results may be in agreement with this hypothesis that during fatigue, calcium is deposited with P_i in SR and therefore, calcium release is limited.

A.1) Pi And the cross-bridge force production

According to current models of cross-bridge force production, myosin heads first weakly and then strongly communicate with the act in filament. Then P_i is released and probably causes a further increase in the generation of force[10].This means that the transition to a strong cross-bridges situation is restrained due to the increased P_i and when Pi is increased through fatiguing stimulations, the cross-bridges are less likely to be in a strong force position. Consistent with this, researches on the skinned fibers indicate that maximum force is reduced in the presence of increasing P_i[11]. Studies on skinned fibers of mammals typically have been done at temperatures much lower than the live body and P_i-induced inhibition in cross-bridge force production is reduced with the increased temperature [12].

Study of Pi effect on the cross-bridge force production in intact muscle fibers is difficult since it has been proved that changing myoplasmic P_i with entering other metabolic changes is hard. An Experimental model which is used in this case is the genetically altered mice that completely lack CK in skeletal muscle ($CK^{-/-}$mice). Skeletal muscle of $CK^{-/-}$ mice during resting shows high concentration of myoplasmic P_i and there is no significant accumulation of P during fatigue. $F_{ca,\ max}$ of fast-twitch fibers of $CK^{-/-}$mice is significantly lower than Wild mice and it would be justified by the decrease of cross-bridge force induced by P_i [13]. Furthermore, it was observed that 10-20 percent reduction in $F_{ca,max}$ in $CK^{-/-}$ fibers after about 10 fatiguing tetanus in fast-twitch fibers is attributed to the increase in P_i[13]. Even after 100 fatiguing tetanus, force in $CK^{-/-}$ fibers was not affected while under the same conditions the force in the fibers of wild mice was reduced to 30% less than the initial force. Further support for reciprocal relationship between myoplasmic P_i concentration and force generation in intact muscle cells comes from experiments in which the decrease in myoplasmic P_i is accompanied by an increase in force generation [1].

Overall, the increase in myoplasmic P_i restrains the force generation with a direct impact on the performance of cross-bridges, and it would be a possible mechanism to explain the reduction in tetanic force production that happens in the early stages of fatigue in the fast twitch fibers. This large reduction of P_i in Cross-bridge force production in mammalian muscle probably will be small at physiological temperature (about 10% of maximum force).

A.2) Pi and myofibrillarCa^{2+} sensitivity

Changes in cross-bridge performance may have effect on the relationship of the force and $[Ca^{2+}]_I$ due to a complex interaction of myosin cross-bridge connection and activation of thin act in fibers [14] and reduction of myofibrillar Ca^{2+} sensitivity frequently has been observed in fatigued skeletal muscle [15]. Experiments using skinned fibers have shown any increase in P_i causes to reduction in myofibrillarCa^{2+} sensitivity [16]. Interestingly, the inhibitory effect of P_i on myofibrillarCa^{2+} sensitivity at 30 C° is more than 15 C°[17], on the contrary, the inhibitory effect of P_i on the cross-bridge force production in concentration of the saturated Ca^{2+} is lower at higher temperatures [18]. Results obtained from non-fatigued $CK^{-/-}$fibers (Which indicate high concentration of myoplasmic P_i during resting) suggest that the myoplasmicCa^{2+} sensitivity is reduced due to P_i[13].

In summary, fatigue induced increase in P_i can reducemyofibrillarCa^{2+} sensitivity which may have a significant effect on force production at the last stage of exhaustion in which tetanic $[Ca^{2+}]_i$ is reduced.

A -3) P_i and increase in tetanic $[Ca^{2+}]_i$ at early fatigue

Usually, at the beginning of fatigue due to tetanic stimulation, an increase in tetanic $[Ca^{2+}]$iis seen. This increase was not observed in $CK^{-/-}$muscle fibers, but after CK injection to these fibers, this increase appeared again [19]. By the time CK was blocked pharmacologically, the same results were observed in Xenopus fibers [20].These findings show key role of CK and P_i raise in the initial increase in tetanic $[Ca^{2+}]_i$. There are several Mechanisms in which CK activities and P_i rise can cause an increase intetanic $[Ca^{2+}]_i$. First, P_i raise may reduce myoplasmic ATP buffering because of the reduction of Ca^{2+}binding to troponin C due to decreased strong cross-bridge attachment[11], but apparently this mechanism has a little importance in skeletal muscle[14]. Second, P_i Can act on Ryanodine receptors (RyR) and increase the rate of SR calcium release. Hence, it has been shown that P_i raise increases the probability of Ry Rbeing open and the amount of calcium-induced calcium release in SR vesicles and skinned fibers [21]. But it has been shown that P_i reduces caffeine-induced and depolarization-induced calcium release from SR in skinned skeletal muscle fibers of rat in an Mg^{++}dependent way [22]. The causes of these contradictory results are not clear, but perhaps differences in laboratory conditions such as activation method of SR and the presence or absence of RyR dependent proteins could be involved[22]. Third, high levels of P_i may inhibit or even reverse SRCa^{2+} pump [23] that at least in the short term can increase tetanic $[Ca^{2+}]_i$[24]. Furthermore, removing PCr prevent SR calcium consumption in skinned fibers [25], that is possibly due to the reduction of ATP buffering through the CK reaction which causes to the reduction of ATP/ADP · P_i ratio in the vicinity of SR calcium pumps [22]. One of the important roles of CK and P_i in SR calcium pump is confirmed by the initial increase in tetanic$[Ca^{2+}]_i$ during fatigue that is followed by significant increase in domain sequence of titanic $[Ca^{2+}]_I$ during the rest in the normal fibers, but notin $CK^{-/-}$fibers[13].

The result is that a CK operating system and P_i accumulation is essential for the initial increase in titanic $[Ca^{2+}]_i$ during fatigue, but the exact involving mechanism(s) is(are) not clear.

A. 4)P_i and reduction in titanic $[Ca^{++}]_i$

After an initial increase, titanic $[Ca^{2+}]_i$ is reduced consistent with fatiguing stimulation, and this is followed by reduction inmyofibrillarCa^{2+}sensitivity which causes to rapid and final reduction in tetanic force. It seems that increase in CK and P_i play a central role in this reduction because in the fibers that CK was inhibited pharmacologically[26]or genetically ($CK^{-/-}$ muscle) [27], the rapid final reduction in tetanic force was delayed. In addition, CK injection into the $CK^{-/-}$fibers leads to normal changes in tetanic $[Ca^{2+}]_i$ during fatigue (initial increase and then reduction) [19].In this opportunity, two mechanisms will be discussed again by which an increase in P_i may reduce Ca^{2+}release: P_i induced inhibition of Ry R and Ca^{2+}-P_i sediment in SR, which decreases free available Ca^{2+} for release.

Experiments on skinned fibers indicate inhibitory effect of P_i on caffeine and SR depolarization induced calcium release [22]. This inhibitory effect appears to be different from P $_i$effect on the SR calcium pumps and Ca^{2+}-P_i sediment in the SR which is attributed to the P_i effect on the SR calcium release mechanism [22]. Interestingly, the inhibition due to the effect of P_i is dependent to the Mg^{+2} changes within physiological range. In a way that inhibitory effect of Myoplasmic free $[Mg^{2+}]$in fatigued fibers (≈1/6mM) was more than resting mammalian muscle fibers (≈0/8mM) [24]. Therefore, theMg^{2+}dependent inhibition in the calcium release from SR due to P_i is constant during fatigue under normal conditions with inverse relationship between tetanic $[Ca^{2+}]_I$ and $[Mg^{+2}]_i$ and completely disappears after pharmacological inhibition of CK[26].

Probably Ca^{2+}-P_i sediment in SR is an important infrastructure mechanism for reducing tetanic$[Ca^{2+}]_i$ in the last phases of fatigue[28]. The causal theory of this issue is that free $[Ca^{2+}]$in SR ($[Ca^{2+}]_{SR}$) is approximately equal to 1mM and solubility of the resulting Ca^{2+}-P measured in the laboratory condition is approximately 6mM [29]. During fatigue when myoplasmic P_i begins to rise, some of the P_i ions enter SR and when the solubility of the resulting Ca^{2+}-P rises further, sediment occurs and $[Ca^{2+}]_{SR}$ reduces [29]. This reduction in the releasable Ca^{2+}pool in SR can lead to reduction in Ca^{2+}release from SR (depending on Ca^{2+} load in SR)[30].

Occurrence of Ca^{2+}-P_i sediment in SR has not been shown directly, but there are indirect evidences which has been obtained from the experiments done on the skinned fibers with healthy SR exposed to the solutions containing high P_i[22] and also from intact fibers of mouse exposed to P_i injection [28]. In one of the recent studies, P_i was injected into the fast-twitch muscle fibers of non-fatigued mice which leaded to a reduction in tetanic$[Ca^{2+}]_i$, faster uptake of calcium by SR and reduction in resting$[Ca^{2+}]_I$ which all are in agreement with reduction in$[Ca^{2+}]_{SR}$. Also, several studies have supported sediment mechanisms of Ca^{2+}-P_i. First, $[Ca^{2+}]_I$ decreases in all muscle fibers in response to high doses of caffeine in fatigued mice [31]. These compounds directly stimulate Ca^{2+}releasing channels of SR, and reduced response is an indicator of reduced available Ca^{2+} pool for release. Second,$[Ca^{2+}]_{SR}$ was reduced during the

fatiguing stimulation of toad muscle fibers and then returned to the initial state [32]. This return in $[Ca^{2+}]_{SR}$ to the initial state, occurred even in the absence of $[Ca^{2+}]_o$ indicating that the Ca^{2+}has not exited from cell during fatigue. On the other hand, return to the initial state of $[Ca^{2+}]_{SR}$ was blocked through mitochondrial inhibition by cyanide, which indicates it is related to aerobic metabolism.

While lots of findings support the important role of the Ca^{2+}-P_i deposit during fatigue, there are also findings that are not consistent with this mechanism [33]. For example, fatiguing stimulation creates the dramatic initial increase in P_i while reduction in tetanic $[Ca^{2+}]_I$ happens with delay. Moreover, in mice fast-twitch fibers, reduction of tetanic$[Ca^{2+}]_I$ temporarilyis associated with increase in cytosolic $[Mg^{+2}]$ which is perhaps originated from the pure decomposition of the ATP [34], and it is not clear why the Ca^{2+}-P_i precipitation in SR should show temporary correlation with increase in $[Mg^{+2}]$ / decreased[ATP]. However, membrane of SR, has chloride channels with small conductance, which may transfer P_i[35]. These channels' probability of being open increases in low ATP which fits with the finding that Pi entering to SR of skinned muscle fibers is inhibited by ATP [36], although others have not observed this dependency on ATP [28]. Additional uncertainties about the importance of Ca^{2+}- P_i precipitation in SR during fatigue include: the necessary concentration of Ca^{2+} and P_i for precipitation in SR should be more than simple salt solutions, Ca^{2+}-P_i may have a different shapes [1], and unstable compounds dissolve rapidly, indicating that by the decline in $[Ca^{2+}]_{SR}$ during tetanic contraction probably there will be more Ca^{2+} available.

As a result, while its clear that P_i raise could reduce tetanic$[Ca^{+2}]_I$ at the end of fatigue, the importance of this subject in various kinds of fatigue and exact involving processes requires more studies.

A.5) creatine supplementation

Creatine has widespread use among athletes at both elite and recreational levels [37]. Creatine supplementation for improving muscular performance has been used for some diseases, such as inflammatory and mitochondrial myopathies and muscle dystrophy. Using a Na^+-dependent transfer in sarcolemma, creatine enters the muscle cell. Inside the cell, creatine phosphorylation happens by CK, and the ratio of phosphocreatine to creatine concentration is related to the energy state of the cell. Surplus Consumption of Cr increases total muscle PCr up to about 20 percent. Creatine supplementation has positive effect on muscle function in short term (activities up to10 seconds) through power exercises while it does not affect performance during long term muscular activity [37]. This issue is related to the fact that PCr decomposition is associated with a relatively large fraction of ATP sources during the first few seconds of intense muscular activity while PCr involvement is less in long term sport activities.

In muscle cell level, increased PCr concentration will provide better ATP buffering during intense activity and will reduce the ADP raise which may decelerate cross-bridge cycling and SR calcium pumping. [25]. So, for a little time much amount of higher power output can be maintained. Also, the PCr to Cr ratio is osmotic active and creatine supplementation in short term generally is associated with increased of body weight due to water accumulation in muscle cells [38]. Increased water content in muscle cells may improve function by increasing myofibrillarCa^{2+} sensitivity and $F_{Ca, Max}$[38].

For fatigue at the cell level, it can be expected that increased PCr loading causes significant reduction in contractile function in intense activities due to the higher rise in P_i(which is the main reason of myofibrillar and SR performance inhibition due to the fatigue). However, it should be noted that the cross-bridge force generation and myofibrillarCa^{2+} sensitivity are linearly reduced with $[P_i]$ logarithm [11], in other words at the beginning of the fatigue that $[P_i]$ is low, depressive effect of P_i raise is high but by the fatigue development and $[P_i]$ reaching to high levels, this effect becomes less and less. Dependency of $[P_i]$ to the fatigue-induced changes in SR calcium handling is probably complex and current understanding of the involved processes, is very limited in order to predict the impact of increased PCr loading on these processes. It should be noted that the positive effect of increased PCr has been observed in short term exercises, in which inhibitory effects of energy metabolites on SR calcium release is limited.

B) Lactate and H^+

Accumulation of lactic acid, has been suggested as a major cause of muscle fatigue since long time ago [15]. As it is known, lactate and H^+ are produced in muscle during intense exercise and in humans, the concentration of intracellular lactate may reach to 30mM or more and intracellular pH (pHi) decreases to 0.5pH unit[1]. A close correlation has been observed between muscle force reduction and increased intracellularlactate and H^+ concentrations. However, this correlation disappears in many cases and even though, H^+ level increase may reduce the performance to somewhat, it has been recently shown that the unfavorable effects of H^+ has been exaggerated and some of its favorable effects, has been ignored[1].

Mechanisms that lead to the reduction of maximum force have focused on two factors of metabolism: acidosis and increased P_i(which is a secondary factor occurring after PCr decomposition). However, several findings suggest that in normal conditions (in vivo), acidosis does not lead to a reduction in maximum force. First, at the beginning minutes of recovery after static contraction to fatigue, force quickly returned to its initial level in spite of acidosis[7]. Second, when rat fibers were studied at physiological temperature, acidosis had only an insignificant impact on maximum force production, so primarily the force loss is due to the accumulation of P_i. However, in animal studies and human muscle, force reduction had a close relationship with double proton P_i comparing total P_i.

Thus, acidosis intensifies the P_i-induced failure in the force. Furthermore, acidosis inhibits ATP resynthesis. Thus, indirectly, it affects the accumulation of hydrolysis by products of ATP (ie, ADP and P_i) in the force generation[7].

B-1), lactate

No relationship has been found between blood lactate and muscle fatigue among people who do sports in different intensities, [1]. Adding lactate to out of the muscle leads to slight reduction of titanic force[39], most part of this effect is not due to lactate uptake, but because of the increased extracellular osmolality and subsequent movement of water to out of muscle fibers which increases intracellular ionic strength and this intracellularionic increase, has a direct inhibitory effect on force generation[40]. Experiments on skinned muscle fibers in constant ionic load have shown that lactate has relatively small impact on force generation even in concentrations higher than 50mM that causes less than 5 percent reduction in $F_{ca,\ max}$ which has very little impact on the sensitivity to calcium[41]. Furthermore, although lactate has a little inhibitory effect on direct activation of SR calcium release due to caffeine or Ca^{+2}, the study of skinned fibers which maintained their functional excitation-contraction coupling suggests that activation of voltage-sensitive Ca^{+2} release is too little even if it has been affected and tetanic and twitch forces are unchanged in the presence of 30mM cytoplasmic lactate[41]. Also, it has recently revealed that the increase in intracellular lactate concentration does not cause to inflation of fiber which is associated to compensatory effects of intracellular H^+ changes in the number of active osmotic particle[42]. Moreover, the gradual accumulation of extracellular lactate in the surrounding of the muscle fibers in vivo during exercise may prevent any tendency for intracellular lactate to trigger vacuolation in the T system[43]. In summary, it seems that the accumulation of intracellular lactate itself is not a major factor in muscle fatigue.

B-2) pHi and muscle fatigue

Muscle pHi is about7.05 at resting in human and, after exhaustive exercise may reduce to less than 6.5[1]. However, in other cases, pHi only reaches to about 6.8 or 6.9 at the point of exhaustion[44] which indicates that muscle fatigue in human, often occurs without a large increase in$[H^+]_i$. Similarly, in rat gastrocnemius muscle which were stimulated in vivo, titanic force reduced by about 60 percent, although pH_i only reached to 6.9[45]. In monitoring of pH_i in isolated mice fast-twitch fibers with fluorescent markers under repetitive stimulation of intermittent, similar findings have been obtained[46]. In addition, people suffering myophosphorylase deficiency are unable to use muscle glycogen and their muscle would get tired earlier than normal people, which this early fatigue occurs with no changes in pH_i[1]. Thus, it is clear that factors other than increased $[H^+]_I$ are also involved in muscle fatigue.

At the beginning of exercise or muscle stimulation, pH_i may initially increase to 0.1 unit due to consumption of H^+ during the PCr breaking down [44] and, conversely, after the end of exercise pH_i can reduce to 0.1 unit more since PCr gets re-synthesized [1].importantly, in the case that pH_i reaches to the lower levels in fatigue muscle, as soon as the exercise or stimulation is finished, force generally returns to its initial state much faster than pH_i[47], which shows low pH is not perse the only reason for force reduction. Furthermore, decelerating pH reduction during stimulation did not reduce the size of fatigue development in frog muscle fibers[48].

When pH_i is reduced experimentally (eg, by increasing the concentration of CO_2), there is a weak correlation between pH reduction and muscle contractile performance dysfunction particularly in mammalian muscle fibers at physiological temperature. Ranatunga[49]observed that the twitch and tetanic force in EDL fibers not only did not decrease but also increased at the temperature of 30-35 C° in acidic pH_i (e.g., about 6.5). In cat muscle in the temperature of 37C° and under living tissue, reduction of muscle pH_i to about 6.3 by hypercapnia reduced thesoleus and triceps brachial maximal tetanic force only about5-10percent[50]. In mice individual intact fibers at 32 C°, the reduction of pH_i from 7.17 to 6.67 caused to 10 percent reduction in maximum titanic force and insignificant reduction maximum shortening velocity of the muscle [51]. In all these cases, decreased pH_i to levels observed in the fatigued fibers, caused to a relatively low reduction in titanic force. In the frog fibers similar conclusions were obtained too [52].

B.3) The effect of low pH on SR voltage sensors activation induced Ca^{+2} release

Another path that was previously thought to reduce the force production was inhibition of calcium release from SR. Low pH reduces the direct activation of Ca^{+2} release channels in response to caffeine and calcium[53], but activation

of voltage-dependent calcium release(common physiological mechanism) and also activation of voltage sensors themselves in the lowerp H like 6.2 would not be affected significantly[54].

B-4) The effect of low pH on calcium binding to TnC and SR pump

Low pH, reduces the calcium sensitivity of the contractile apparatus[54], perhaps because H^+ competes with Ca^{2+} for binding to troponin C. It was often assumed that it would have a destructive effect on muscle function. However, low pH, reduces Ca^{+2} affinity for connecting to other sites of muscle fibers, especially to the SR calcium pumps. Thus, although affinity of troponin C (TnC) to calcium may reduce but final amount of calcium binding to TnC may not reduce. A single action potential of release triggers releasing large amounts of calcium from SR(approximately 20% of the SR calcium content or about the 230μM Ca^{2+} to the total volume of the muscle) [30] and an increase in the concentration of free calcium in cytoplasm is only a very small portion of this amount because large portion of Ca^{+2} is connected to TnC, the calcium pump and other sites [30]. Lowering the pH from 7 to 6.3 reduces calcium pump affinity in SR more than five times (0.14-0.72 μM)[55], which is significantly greater than the effect on the contractile apparatus (less than 2 times of decrease in affinity for calcium) [56]. As a result, the amount of calcium available for binding to TnC is likely to be higher at acidic pH. In addition, a further reduction in affinity for calcium and the calcium uptake amount by sarcoplasmic pump in acidic pH leads to an increase in resting free calcium concentration [57] which probably helps maintaining or even increasing the amount of various occupied sites for binding to cytoplasmic calcium. As a result of all these influences, the concentration of free calcium in cytoplasm reaches to the values higher than normal pH during acidic pH levels [57] and response force to twitch actually increases[49], and minimum tetanic force grows up faster in frog muscle [58].

In summary, in comparison to what was previously thought under physiological conditions, low pH has slightly lower inhibitory effects on contractile apparatus activation and calcium release, and its effects on SR calcium pump is in the interest of force generation.

B-5) the effect of low pH on membrane conductance

As it was noted previously, low pH_i can have beneficial effects on muscle function by maintaining excitability. Nielsenet al[59] showed that lowering pH_i to around 6.6 helps to overcome the loss of excitability that occurs during membrane depolarization in both fast-twitch and slow-twitch fibers in mammals. This happens because lowering pH_i reduces Cl^- transmission power of membrane in T system almost couple of times, which allows the potential action to spread both in the surface and membrane of the T system and thus, contraction begins[59]. Kristensenet al[60] confirmed this finding that lowering pH_i by adding extracellular lactic acid helps bringing back excitability in depolarized muscle of mammals, but they stated it happens inactive muscle because during fatigue in the vitro, force reduction rate was not affected. However, in those experiments, deep muscle fibers may be faced with hypoxia due to high amount of exicitation and limited distribution of oxygen, which could increase the rate of fatigue, so it hides the beneficial effects of low pH_i[61]. Furthermore, it is likely that decreased pH_i values lower than its usual values during fatigue would have no more beneficial effect on the excitability. Experiments in which lactate was injected to the animals while muscles forced to get fatigued by nervous or direct stimulating of muscle, it has been revealed that the presence of extracellular lactate which probably had reduced the pH_i, decreased inability in sarcolemma excitability and improved force generation congruent with the acting on Cl^- transferring power [62].

B-6) The effect of low pH on fatigue rate

It has been indicated that in isolated muscle fibers, reduction of pH_i from 7.18 to 6.77 does not reduce initial tetanic force or muscle fibers fatiguing speed though final pH_i was low at the point where the force fell down to 40% (6.46pH_i in contrast to 6.91 for control group) [46]. In fact, in the acidic conditions 98 ±18 tetanic stimulation was necessary to force fell down into 40%, while in control group 63 ±10 stimulation was needed which suggests that the rate of fatigue development is bellow in the low pH_i. This effect may be partly due to better excitability of T system [63] or substantially due to pH-dependent reduction in ATP consumption and thus reduction in cross-bridge cycle [46]. These findings provide evidence that low pHi does not accelerate fatigue speed in muscle fibers through decreased glycolysis. Similarly, in humans, pH i reduction that happens in exhaustive exercise, does not inhibit glycolysis and glycogenolysis[64] and studies by electrical stimulation in humans have shown that although glycogenolysis and glycolysis rate are reduced during pH i drop From 6.7 to 6.45, however the activity is meaningfully maintained [65].

Finally, there have been many studies that investigated the impact of whole body pH manipulation on exercise performance and some of them reported negative effects of acidic environment and positive effects of alkalosis on performance [66] but others did not reported that. However, the body pH manipulation can have some effects on blood O2 saturation and release, local vascular function, CNS drive and other factors. Studies with lactate injection into rats hind limb indicated that pH reduction has negative effect on muscle function, but this reduction in performance was due to some direct effects of low extracellular pH and not due to reduced pHi[1]. Data resulted

from intravenous injection pressure showed that this effect is probably due to the acidification that disturbed local control of blood flow in the muscle vascular bed. Similarly, alkalosis in humans can delay the onset of fatigue [67], but such alkalosis may lead to decreased extracellular potassium concentration which can have some effects on membrane excitability. Interestingly, while preventing such changes in the concentrations of potassium and other effects, alkalosis does not improve muscular performance [53]. Finally, it has been made clear that increasing muscle carnosin by beta-alanine supplementation delays onset of fatigue during exercise which is due to the increase of pH buffering by the carnosin[68], but positive effects of carnosin is generally because it dramatically increases the Ca^{2+} sensitivity of the contractile apparatus [68].

As a result, high $[H^+]_i$ is not a major cause of muscle fatigue because its direct impact on force generation is low.

B-7) The effect of acidosis on deceleration of relaxation

When individual fibers were exposed to 30% increase of CO_2, relaxation decelerated to a significant extent, but when acidosis was prevented through the inhibition of glycolysis by iodoacetic, relaxation was not decelerated. It was apparent that during acidosis, re-uptake of calcium in SR is suppressed which is probably performed through inhibiting the proliferation and formation of phosphorylated enzyme mediator [7]. All these results are consistent with the hypothesis that acidosis reduces the relaxation rate and also its function is related to Ca^{2+}-ATPase activity reduction in SR during acidosis. In vivo experiments are in agreement with this concept. During intense exercises relaxation is extremely decelerated with lactic acidosis, but during long distance cycling with moderate intensity up to the fatigue, when the level of lactic acid in the muscle remains low, relaxation rate remains unchanged. McArdle disease patients lack glycogen phosphorylase and during contractions do not encounter with acidosis. Contraction up to the fatigue decelerates relaxation in these patients, but not to the extent which is seen in healthy people. In addition, they also recovered more quickly [7]. All in all, it is concluded that at least two processes are involved in decelerating the relaxation, one independent and another dependent on acidosis.

C) ATP and Mg^{+2}

It seems that when ATP consumption overtakes its resynthesis, the levels of ATP, ADP, IMP and $[Mg^{2+}]_i$ change during exercise. During intensive fatigue ATP may reduce from 7 to 1.2 mM and PCr may reduce from about 30 mM to 25 mM, while probably ADP may increase from 10 μM to more than 200 μ M, IMP from the immeasurable values to 5 mM and finally $[Mg^{2+}]$ i from 1 mM to 2 mM or more. in slow-twitch fibers, cytoplasmic [ATP] reduction will be very little if there is any [69]. In fast-twitch fibers in certain areas of muscle cells these changes may be even more than the mean of cytoplasmic changes. In this part consequent changes in contractile apparatus and E-C coupling will be considered which may be involved in the fatigue onset.

C -1) contractile apparatus

Maximum force production in skinned fibers does not decrease unless ATP concentration reaches to less than 20μ M and maximal force increases almost 10% when ATP reaches to 0.5-1 mM and also ADP and AMP reach to less than mM[70].

Ca^{2+} Sensitivity is not changed by reduction of ATP concentration to 0.5 mM, but Ca^{2+} sensitivity is increased if ADP level be less than mM[70]. One of the first recommendations was that the IMP accumulation may cause fatigue [15], however, 3 mM IMP had no effect on maximal force and caused little increase in calcium sensitivity [71]. Increasing the concentration of magnesium to 3 mM has no effect on maximal force or shortening velocity [72] but cause a significant reduction in calcium sensitivity (1.6 times increase in required calcium concentration for generating 50% of maximal force) [71]. In fast-twitch fibers, the combinational effect of these changes may be a slight increase in maximal force but remarkable reduction in Ca^{2+} sensitivity for force production. These effects may be other than the changes which are seen during increased P_i[53].

C-2) Pumping and leakage of Ca^{2+} from the SR

studies done on the skinned fibers show that when the ATP concentration decreases up to 0.5 mM, tetanic relaxation rate reduces up to 2.5 times (at low concentrations and a constant ADP) [72]. This is not due to the effect on the contractile apparatus but probably due to a reduction of Ca^{2+} uptake by the SR calcium pumps. Although cites of ATP breaking down on the SR pumps have a very high affinity, ATP has also a regulatory function on these pumps. For example, when ATP concentration decreases from 5 to 0.25 mM, SR pumps affinity to calcium is reduced almost 10 times [72]. This change may enable ATPase to continue to pump calcium despite a dramatic reduction in free energy resulting from ATP hydrolysis because only one calcium ion is transferred per each hydrolyzed ATP molecule, but it means a reduction in energy efficiency of the process. If affinity of SR Pump to Ca^{2+} is decreased with acidic pH (pH=6.3), decreased ATP concentration would have a negligible impact [73]. In mammals fast twitch fibers, increased ADP concentration from 10M μ to 0.2mM causes to small reduction in calcium pumping and two-times increase in leakage of Ca^{2+} from the SR because pumps get loosed [74]. Such changes reduce the calcium

uptake rate and increase resting calcium concentration in intact fibers. If PCr reaches to low levels, increased Ca^{2+}leakage from SR will be observed even in presence of 5-8 mM ATP [74], indicating that if PCr is depleted, local ADP concentration will be difficult to control in areas close to the calcium pump. Interestingly, in the slow-twitch fibers, increased ADP concentration has little effect on the rate of pumping and SR leakage [75]. Finally, if the Mg^{2+} concentration raises from 1mM to 3 mM or IMP level reaches to 3 mM, Ca^{2+} uptake will be reduced in the fast-twitch fibers [75].

In summary, studies on skinned muscle fibers suggest that decreased [ATP] and [PCr] can reduce the SR Ca^{2+} pumping and increase leakage that will increase $[Ca^{2+}]_i$ level as we see in fatigue. Under some circumstances, decreased ATP concentration may reduce relaxation of fatigued muscle.

C-3) SR calcium releasing

Calcium release channels in mammals skeletal muscle (RyR) is stimulated by ATP binding in cytoplasmic regulatory cites, and ADP and AMP act as its weak agonists but IMP do not [72]. Also, the calcium release channels are inhibited by Cytoplasmic Mg^{2+}[72] and in resting concentration of Mg^{2+} (approximately 1mM) remain closed unless they are directly activated by voltage sensors. By increasing $[Mg^{2+}]$ From 1 to 3 mM, voltage sensors induced calcium release is reduced by approximately 40% [72]. By reducing [ATP] to 0.5 mM, voltage sensor induced calcium release is reduced approximately 20% and when increased $[Mg^{2+}]$ is accompanied by reduced [ATP], that reduction will be more, and it would be more even if there are AMP and adenosine which are the products of ATP hydrolysis [69]. As a result, it is likely that the combination of these factors reduce voltage sensor induced calcium release significantly. Reduced tetanic $[Ca^{2+}]_i$ observed in $CK^{-/-}$ mice at the beginning of intense stimulations is attributed to inhibitory effect of increased $[Mg^{2+}]$ and decreased [ATP] on calcium release [27], but it is obvious that these are not only metabolic factors that are involved in reducing calcium release during fatigue [26].

In short, during intense exercises, the triad junction may play key role by sensing depletion of cellular [ATP] levels and responding by reducing Ca^{2+} release. This phenomenon reduces the consumption rate of ATP by reducing the cross-bridge cycle and calcium uptake by SR (Two major sources of ATP hydrolysis). The cost of this task is reduction in power output or in other word, muscle fatigue but, the benefit is preventing complete ATP depletion and subsequent rigor and cell damage.

C-4) ATP and deceleration of relaxation

Measurement of phosphorus metabolism with MRS in vivo indicates that relaxation rate is positively correlated with adenine nucleotides phosphorylation and Gibbs free energy changes induced reduction in ATP hydrolysis, justifies the reduction of the relaxation rate [7]. Isolated SR vesicles measurement provides evidence that increased local ADP prevents calcium pumping and also ATP resynthesis through PCr system in the vicinity of ATPase is necessary to remove Ca^{2+}. In addition to removing Ca^{2+} from cytosol, the relaxation rate depends on the cross-bridge detachment. The importance of this factor varies depending on different species and conditions. Detachment of cross-bridges apparently is the main factor in determining the degree of relaxation in mice muscle, whereas in human skeletal muscle the degree of relaxation is more dependent on the Ca^{2+} removing [7].

D) Glycogen

In skeletal muscle, glucose is stored as glycogen and these reserves are important source of energy during different kinds of muscle activity. Direct correlation between muscle glycogen concentration and time to fatigue during relatively intense exercises(60-80% of VO_{2max}) first was shown by Hermansen et al [76]. Their findings were confirmed in several studies, and the relationship between glycogen depletion and fatigue during relative intense activity is now fairly proved. After prolonged exercise (over two hours) without supplementation of carbohydrate, liver glycogen is depleted and blood glucose is reduced. Since glucose is the main substrate of neural fiber, hypoglycemia disturbs CNS performance and the exercise (central fatigue). The importance of carbohydrate supplementation in the prevention of hypoglycemia and development of performance is well established [7]. Although fatigue can cause reduced motor units recall and as a result cause reduction in force [77], but relationship of glycogen depletion and decreased force during fatigue is not well understood [1]. Studies which measured Energy and $[Ca^{2+}]_i$ simultaneously, tried to elucidate the mechanisms of relationship between fatigue and low glycogen. In an initial research in FDB fast-twitch fibers which were made fatigued by frequently tetanus, muscle glycogen content reached to 25% of the control [78]. When recovery had done in the absence of glucose, glycogen level did not return to the original level and muscle fibers became more easily fatigued on the next run. Studies have shown that the reduction in tetanic force was accompanied by Ca^{2+} reduction during fatigue and when glycogen was not restored with glucose, the reduction was faster [78]. Similar results were obtained in toad muscle fibers that were made fatigued by frequently use of tetanic stimulation in the absence of glycogen [79]. Also, the EDL fast-twitch fibers of mice were made fatigued with repeated tetanic stimulation and they were given two hours to recover with high, normal and zero extracellular glucose and again they became fatigued. Fibers that were recovered with zero

glucose had lower levels of glycogen (50% of control) and at the beginning of the second round of stimulation became fatigued faster than others. The rapid fatigue affects tetanic force and $[Ca^{2+}]_i$[80]. Therefore, low levels of glycogen is accompanied by early fatigue development, reduction in tetanic $[Ca^{2+}]_i$ and force.
The relationship between glycogen reduction and low concentration of $[Ca^{2+}]_i$ may be that glycogen provides needed acetyl coenzyme A for tricarboxylic acid (TCA) cycle or maintains high levels of TCA mediators[80]. Alternatively, the relationship between low glycogen and abnormal SR calcium release directly has not been attributed to key role of glycogen in the energy metabolism. This result arises from a study that showed in toad skinned muscle fibers, ability to respond to T tubes depolarization has close relationship with muscle glycogen content [81].In above mentioned experiments with skinned fibers, ATP and PCr existed in washing solution suggesting that glycogen structural role was greater than its metabolic role. Several similar studies on the EDL skinned fibers of rats has been done which has shown that these mammals fibers had only minor effect of glycogen dependent on ATP And PCr on potential response to depolarization [82].Data from fatigued intact fibers support both the structural and metabolic role of glycogen. Isolated muscle fibers FDB and EDL became fatigued much faster by the time that glycogen dropped after recovery by zero glucose which is compatible with both possibilities [78, 80]. However, glycogen depleted cells showed typical changes in other parameters related to fatigue (increasedmyofibrillarCa^{2+} sensitivity and maximal force, slower relaxation and increased resting $[Ca^{2+}]_i$) which are commonly attributed to metabolic changes [78, 80]. On the other hand, fatigue in toad muscle fibers was accompanied by rapid reduction in SR Ca^{2+} sources, while these sources were not reduced after a rapid fatigue in glycogen free cells [79]. Therefore, this information about toad fibers showed different mechanisms in the reduced SR Ca^{2+} release caused by fatigue in normal and glycogen free cells.

As a result, glycogen depletion during prolonged and exhaustive exercise could be involved in the onset of fatigue due to reducing calcium release from SR. Bonding mechanism of Low glycogen and early failure of the calcium release from SR has remained uncertain.

E) Reactive oxygen species (ROS)
Muscle contraction and its dependent increase in renewing the energy is mainly dependent on forming excessive reactive oxygen species (ROS) [7]. Experiments on rat diaphragm muscle fibers show that ROS affects the force-frequency relationship [7]. In rat individual fibers during submaximal tetanus, myoplasmic$[Ca^{2+}]$ was not affected by ROS, while the myofibrillarCa^{2+} sensitivity changed in a time-dependent method. In conclusion we can say ROS cause an increase in low frequent fatigue by reducingmyofibrillarCa^{2+} sensitivity[7].Experiments in humans also support the conclusion that ROS leads to low frequent fatigue. When non-specific antioxidant was used in human subjects (N-acetylcystein), power output had increase during fatiguing exercise in low-frequency stimulation (10 Hz), but not in high-frequency stimulation (40 Hz). These findings indicate that oxidative stress affects contractile force, but its mechanism is not clear [7].

F) Hormonal basis of fatigue
Studies have shown that an exercise up to the fatigue causes to considerable reduction in testosterone level and its reduction rate depends on the intensity, duration and the distance of the exercise [83-86]. Moreover, this reduction is not dependent on the type of exercise and is observable in all sorts of endurance activities such as running and rowing [87]. Although, all of the researches have not reported lower levels of testosterone at the end of exercise, but all have agreed on this point that testosterone concentration reduces at least half an hour after the end of the activity[88].

Also, plasma prolactin had more increase after exercise in the form of running on the treadmill comparing to non-athlete individuals that may be provide by cortisol stimulation [89]. Apparently the prolactin rate was increased after exercise up to the fatigue and remained high until 30 minutes after the end of exercise [90-93].It seems that serotonin secretion stimulates the prolactin receptor and causes to more secretion of prolactin from pineal gland. This hormone is made in acidophilic cells of anterior pituitary gland and after release is controlled by hypothalamus [93].Therefore, after exercises especially endurance one in which central body temperature increases due to increased metabolic requirements, increase in the secretion of prolactin is expectable.

In addition to increased prolactin secretion, it has been reported that Dehydroepiandrosteronesulphate (DHEA-S) significantly increases in marathon runners [94]. After this report several studies reported increase of serum DHEA-S due to exhaustive exercise[84, 95, 96]and even after resting, DHEA-S level remained high. Also continual decrease in DHEA-S levels was observed three hours after resting [84]. DHEA-S increase rate has direct relationship with intensity and duration[84]. The reason of increased DHEA-S level can be associated with steroids induced production of adrenocortical for long time after exercise [97].In general, results of the studies show that continual exercises (exercise up to the fatigue) can cause to increased DHEA-S and decreased testosterone[96].

On the other hand, depletion of adrenal gland hormones as one possibility, and decreased cortisol secretion rate as an another possibility fatigue have been mentioned in the onset of excessive. Stoppage of adrenal glands or hypophysis hormones due to the negative feedback related to various factors, cause to a decrease in energy substrates presence[98]. In addition, we know that perhaps, central inhibition of body reserves recall has relationship with serotonergic neurons in the hippocampus. Increased plasma free fatty acids due to the endurance exercise with reduced branched-chain amino acid (BCAA) of the blood due to its uptake by the muscles indirectly facilitates the entry of tryptophan(responsible enzyme in catalyzing the first reaction in the serotonin synthesis)to brain because tryptophan and BCAA compete with each other for a carrier (albumin) in order to be transferred to brain [99]and so leads to increased serotonin concentration in the pre-optic area and hypothalamus [100]. After electrical coagulation of the hippocampus (where a part of the serotonin is produced), the maximum swimming time increased somewhat, but blood corticosterone concentrations was decreased. This information was observed in the mice that had to swim to the fatigue point [101].The result is that lower than normal rate of adrenal cortex activity, is a result of a regulatory mechanism. Obviously, this regulatory mechanism is related with increased activity of hippocampus serotonergic neurons. It turns out that these neurons inhibit secretory neuron cells existing in the hypothalamus producing corticoliberin (corticotropin releasing factor). Less than normal activity of adrenal cortex causes that the effects of catabolic glucocorticoids become limited during the prolonged exercise. At the same time, decreased adrenal cortex activity reduces the synthesis of epinephrine [101] and thus skeletal glycogenolysis and lipolysis in adipose tissue are reduced. It is noteworthy that inhibition of hypothalamic neuronal secretory cells has relationship with the activity of serotonergic neurons [101]. Exercise induced fatigue can change the responses of hypophysis-adrenal cortex. More fatigue increases the likelihood of the dealy in the hypophysis - adrenal cortex axis. In this case, delay can be developed to somatotropin. As a result, it may be stated that stopping signs of hypophysis-adrenal cortex activity, impaired performance of hypophy seal somatotropin and decreased testosterone levels can be considered as indicators of critical fatigue [101].

As a result, increased serotonin concentration as a result of increased free fatty acids and decreased BCAA levels cause to the triggering the central fatigue and also decreased activity of adrenal cortex which restrict the effect of the catabolic glucocorticoids during prolonged exercise. At the same time, decreased activity of the adrenal cortex reduces the synthesis of epinephrine and as a result glycogenolysis and lipolysis are reduced. It can be stated that the changing serotonin level causes some changes in body hormones that increase the development rate of fatigue.

CONCLUSION

Exercise requires hydrolysis and re-synthesis of ATP. This leads to the production and accumulation of metabolites such as P_i, H^+, ADP, AMP and IMP. It seems that in contrary to the previous assumptions , under physiological conditions , low pH has slight inhibitory effects on the contractile apparatus activation and calcium release, and its effects on SR calcium pump is in fact beneficial for force generation . Therefore, high $[H^+]_i$ itself is not the major cause of muscle fatigue. In contrast, fatigue-induced increase in P_i can reduce myofibrillarCa^{2+} sensitivity that may have a significant effect on force production at the last stages of fatigue, where tetanic $[Ca^{2+}]_i$is reduced. Furthermore, the reduction of [ATP] and [PCr] can reduce SR Ca^{2+}pumping and increase SR Ca^{2+}leakage that as we see in fatigue, this incident leads to increased$[Ca^{2+}]_I$ level and in another hand causes to slowing relaxation of fatigued muscle. All these metabolic changes lead to the stimulation of metabolic/pain receptor afferents (group III / IV) originating from the muscles with byproducts of intramuscular metabolism and their input to CNS is increased parallel with the accumulation of metabolites. This central projection reflexively leads to increased blood flow (vasoconstriction induced increase in blood pressure) and ventilation[9]and gradually decreases central motor output / voluntary muscle activation and triggers central fatigue. Yet, after prolonged exercise (without supplementation of carbohydrate) liver glycogen is depleted and blood glucose is reduced. Since glucose is the main substrate of neural tissue, hypoglycemia disturbs CNS function and exercise performance (central fatigue). Central fatigue can cause to reduction of motor units recruitment and decrease force generation. On the other hand, glycogen depletion during prolonged exhaustive exercise could be involved in the onset of fatigue due to decreased SR Ca^{2+} release. But, the linking mechanism of low glycogen and premature failure of SR Ca^{2+}release remains unclear. Along with all these changes, increased serotonin concentration level as a result of increased blood free fatty acids and reduced BCAA, triggers central fatigue and leads to decrease dactivation of adrenal cortex lower than normal, which restricts the effect of catabolic glucocorticoids during prolonged exercise. At the same time, this decreased adrenal cortex activity reduces synthesis of epinephrine and as a result skeletal muscle glycogenolysis and lipolysis. Therefore, the change of serotonin levels causes changes in body hormone that increases the rate of fatigue development. Overall, it can be stated with certainty that fatigue is a complex phenomenon that has many underlying factors and for its better understanding all the factors must be studied together.

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