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Effects of a 4-Week Vitamin B6·B9·B12 Supplementation on the Muscle Recovery and Muscular Function Induced by Acute Eccentric EXERCISE

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Abstract

We examined the effect of a 4-week vitamin B6·B9·B12 supplementation on the muscle recovery and muscular function induced by acute eccentric exercise. Twenty-nine subjects were volunteered for this study, and they were randomly assigned to either vitamin B6·B9·B12 supplementation group(n=14) or placebo group(n=15). Body composition was measured prior to testing. The vitamin B group was given two tablets of vitamin B6·B9·B12 supplement per day from 4 weeks before eccentric exercise until 72 hours after eccentric exercise, whereas the control group was given two placebo tablets. All subjects performed eccentric exercise using Biodex isokinetic machine after treatment period. They performed a total of 4 sets of eccentric exercise with 12 repetitions per set using their non-dominant arm. Blood samples were taken at pre-exercise, immediately after exercise, and at 6, 24, 48, and 72 hour post-exercise. Maximum isometric force(MIF) and range of motion(ROM) were obtained before and immediately after exercise and at 2, 6, 24, 48, and 72 hour post-exercise. Independent t-tests were conducted to analyze differences between groups in physical characteristics and total amount of work of eccentric exercise. Lactate dehydrogenase(LDH) concentration, MIF, and ROM were analyzed using 2-way repeated measure ANOVA. A main effect for test session and group effect were significant in LDH concentration($p<.05$). A main effect for test session for MIF($p<.05$) and ROM($p<.05$) was significant. In both groups, LDH concentration significantly increased immediately after exercise and at 48, and 72 hour post-exercise compared to pre-exercise($p<.05$). LDH concentration of vitamin B group was significantly higher than control group, independent of test session($p<.05$). MIF and ROM significantly decreased from immediately after exercise to 48 hours after exercise, and 72 hours post-exercise, respectively, in both groups compared to pre-exercise($p<.05$). In conclusion, the results of this study suggest that at least 4-week supplementation of vitamin B6·B9·B12 may not have a positive effect on the recovery of muscle damage and function induced by eccentric exercise.

[Keywords] ***Sport, Lactate Dehydrogenase, Maximal Isometric Force, Range of Motion, Delayed Onset Muscle Soreness***

1. Introduction

Skeletal muscle damage frequently occurs by unaccustomed lengthening-type muscle action(eccentric contraction) and an exercise which is sufficient intensity and duration[1]. Indeed, exercise-induced muscle damage results in focal disruption of fiber ultrastructure including desmin and dystropin, increase in volume and circumference of the limb, decrease in the range of motion and muscular strength, and delayed onset muscle soreness(DOMS)[2][3].

Muscle pain and muscle force loss are the most debilitating symptoms after muscle damage. However, muscle pain is usually not evident for several hours after eccentric exercise in humans, but the loss of muscle force is significantly detectable immediately after eccentric exercise. Mackey et al.(2004)[4] re-

ported that contractile force could be decreased by as much as 40-50%, and full recovery of force may not occur for some weeks after exercise. In the case of non-elite people, muscle pain or strength loss would be related to continue of exercise for their improvement of health. Especially, muscle force loss by muscle damage is closely related with exercise performance in elite sport athletes because the heavy competition schedule and training more than once within a single day are oftentimes their routine. Therefore, maximizing and accelerating the recovery from muscle damage is crucial to maximize their exercise performance[5].

There are variety of methods to treat muscle damage or DOMS, and it was known that ice, ultrasound, nonsteroidal anti-inflammatory medication, massage, and anti-oxidative vitamin are representative[6][7][8]. However, methods such as massage, ultrasound, and electrical therapies have disadvantage of longer time to treat compared other methods like medication usage, despite these methods were effective for alleviating muscle pain and inflammatory response, and also the equipment itself is expensive. In addition, although it was reported that nonsteroidal anti-inflammatory drug might have fast effect on decreased swelling, inflammation, and muscle soreness, this method is not recommended due to the major side effects such as gastrointestinal ulceration and bleeding, hepato-renal dysfunction and organ failure, and skin reactions[9][10][11].

The most economical and simplest way to prevent muscle damage is antioxidant treatment, and the most research has focused on the effects of the antioxidant vitamin C and E. Vitamin C, or ascorbic acid, is a potent water soluble antioxidant vitamin, placed in the cytosolic compartment of the cell[12]. Vitamin C exerts its functions by scavenging reactive oxygen species(ROS) and nitrogen species[13]. As vitamin E is the most important lipid soluble antioxidant vitamin, it is virtually found in most cell membranes[12]. It is known that vitamin E has function for stopping the progression of the lipid peroxidation chain reaction, as well as scavenging ROS such as superoxide and hydroxyl radicals[14]. Although there is still controversial about the benefit from the use of antioxidant to prevent muscle damage, it was proposed that the treatment of these antioxidant vitamins can prevent muscle damage by reducing of reactive oxygen species and increasing membrane integrity[12][15].

However, there is some limited evidence about a protective effect of muscle damage for antioxidant vitamin supplementation[16][17], and the majority of studies have failed to demonstrate a protective effect of for antioxidant vitamin treatment on muscle damage. Although it was suggested that long-term supplementation of antioxidant vitamins over 14 days may reduce symptoms of muscle damage, the effect of vitamin C & E on muscle damage is unclear and more researches are needed.

On the other hand, it is known that vitamin B6, 9, and 12 contribute to the synthesis of DNA and proteins and might stimulate the recovery of muscular function from exercise-induced muscle damage[18][19]. Especially, vitamin B6 plays an important role in amino acid and protein metabolism, and as an exercise-related function of vitamin B6, this vitamin has been reported to be associated with the energy-producing pathways such as glycogen breakdown of the body during exercise[20]. Vitamin B9, also known as folic acid is necessary for the synthesis, repair, and methylation of DNA. In the case of vitamin B9 deficiency, this can lead to impaired function of RNA and DNA, lipid metabolism, and muscle formation[21][22][23]. Lastly, vitamin B12 plays a vital role in the cell growth and development of the human body. Vitamin B12 is also required in DNA synthesis, erythrocytes formation, folic acid metabolism, nervous system development, and protein synthesis[18].

Considering the effects of vitamin B6, 9, and 12 on the synthesis of protein and repair from muscle damage, it could be expected that the supplementation of these B vitamins can contribute to maintain muscle function by muscle damage contributing rapid recovery and repair of muscle contractile and structural proteins. However, there is no study examining the relationship between

the muscle function and vitamin B6, 9, 12 supplementation. With this purpose, the current study examined the effects of vitamin B6, 9, and 12 supplementation during 4 weeks on the indices of muscle damage and muscle functions.

2. Methods

2.1. Subjects

Twenty-nine untrained healthy and college-aged men(aged 22.90 ± 2.16 years, height 175.21 ± 5.88 cm, weight 75.95 ± 10.29 kg) volunteered for this study. It was provided the explanation of purposes, procedures and risk of the study, and written informed consent. All subjects completed questionnaires about using medications and supplements, physical activity, medical history to exclude unqualified person. Subjects were composed of people who did not take drugs or vitamin supplements for at least 3 months and had no experience in surgery on the elbow joint. They refrained from high-intensity exercise and extra vitamin supplementation during this study. This study was approved by the Keimyung University's institutional review board for human subjects.

2.2. Supplementation

Twenty-nine subjects were randomly assigned to either a placebo group(dextrose mixture with sodium chloride; *n*=15) or a vitamin B6·B9·B12 supplementation group(*n*=14). Four weeks before the eccentric exercise, all subjects were given supplements. The subjects of vitamin supplementation group consumed two tablets of vitamin B6·B9·B12(one tablet include 1.5 mg B6, 400 μg B9, and 2.4 μg B12; Garden State Nutritionals, U.S.A) per day from 4-week before eccentric exercise until 3 day after eccentric exercise. The placebo group also took two placebo tablets(one tablet include 450 mg glucose and 200 mg sodium chloride; Fitness farm Inc., Korea) per day during the same period.

2.3. Experimental procedures

Before supplementation, all subjects' weight and body composition were measured using InBody 520(InBody, Korea). All subjects came back to the laboratory with remaining supplements after 4 weeks of supplementation period. Intake rate was determined by given pills versus those remained. The subjects continued to intake supplements during the 3 days after the eccentric exercise.

Subjects reported back to the laboratory after 4 weeks supplementation period in the morning after overnight fast and took a rest at least 15 min before obtaining measurements. ROM was measured first, and ROM was measured using a goniometer, assessing the elbow flexors on both arms by asking subjects to flex and extend their arms at the elbows. A resting blood sample was then taken from an antecubital vein. Before eccentric exercise, a researcher adjusted Biodex isokinetic machine(Biodex Medical Systems, U.S.A) to suit the subject's body, and then recorded machine's position for consistency.

MIF was measured by Biodex isokinetic machine, and each subject performed three MIF at fixed 45° of ROM with their non-dominant arm. After obtaining initial MIF, all subjects performed eccentric exercise using their nondominant arm elbow flexors to induce muscle damage at an angular velocity of $20^{\circ}\cdot s^{-1}$ and 100° of flexion. Eccentric exercise was comprised of 4 sets of 12 eccentric contractions with resting of 60 seconds. Total amount of work was recorded for each set and totaled. In addition, peak forces and decline of forces and ROM were also measured by measuring MIF and ROM at immediately after exercise, and 2, 6, 24, 48, and 72 hour post-exercise.

Blood samples were taken 3ml at pre-exercise, immediately after, and 6, 24, 48, and 72 hour after eccentric exercise protocol via vacutainer containing EDTA and immediately processed. Blood samples were analyzed for serum LDH.

2.4. Blood handling and LDH analysis

The collected blood samples were kept at room temperature for 30 minutes to clot then centrifuged at 3,000 rpm. Separated serum was put into microcentrifuge tubes and

stored at –80℃ until analysis of LDH. LDH concentrations were analyzed by doing request at the Seoul Clinical Laboratories. Serum samples were mixed with R1(Lactate) and R2(NAD+) reagent(Roche, Germany) then read at 340-nm wavelength using HITACHI 7600(Hitachi, Japan) to analyze LDH concentration.

2.5. Statistical analysis

Statistical analysis was used the SPSS statistical data analysis software package(Version 25.0; IMB, U.S.A). Subjects' characteristics and total amount of work were analyzed using an independent *t*-test to compared between groups. To compare the differences between time and groups, LDH concentration(2 x 6), MIF, and ROM(2 x 7) were analyzed using two-way repeated ANOVA. The level of statistical significance was set at a $P < .05$. All data are represented as mean ± SD.

3. Results

3.1. Baseline characteristics

Twenty-nine males were randomly assigned to either a placebo group(*n*=15) or the vitamin B6·B9·B12 supplementation group(*n*=14). All subjects successfully completed this study. Their physical characteristics are presented in <Table 1>. There were no statistical significant differences in physical characteristics at baseline between the two groups.

Table 1. Physical characteristics of subjects.

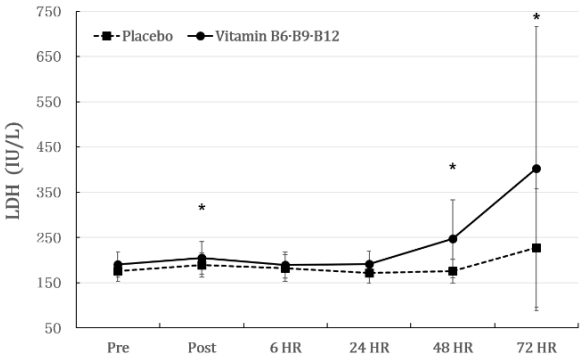
	Placebo group (*n*=15)	Vitamin B group (*n*=14)
Age(yrs)	23.40 ± 1.76	22.36 ± 2.47
Height(cm)	176.87 ±6.46	173.43 ± 4.78
Weight(kg)	77.04 ± 9.70	74.78 ± 11.13
BMI(kg/m^2)	24.60 ± 2.55	24.83 ± 3.39
Body fat(%)	17.28 ± 6.75	19.28 ± 7.47
Skeletal muscle mass(kg)	36.28 ± 3.87	33.89 ± 3.24

Note: Values are presented as mean ± SD. BMI: Body Mass Index. There was no significant difference between the two groups(P > .05).

3.2. LDH concentration

LDH concentration showed significant group($P < .05$) and time effect($P < .05$) <Figure 1>. The vitamin B6·B9·B12 supplementation group had a significantly higher LDH concentration than placebo group, independent of test session. A significant time main effect($P < .05$) occurred for serum LDH concentration, independent of treatment, LDH concentration significantly increased at immediately after exercise, 48 and 72 hour after exercise compared to pre-exercise.

Figure 1. LDH concentration in response to eccentric exercise over time. Values are presented as mean ± SD. *Significant different compared with Pre($P < .05$).

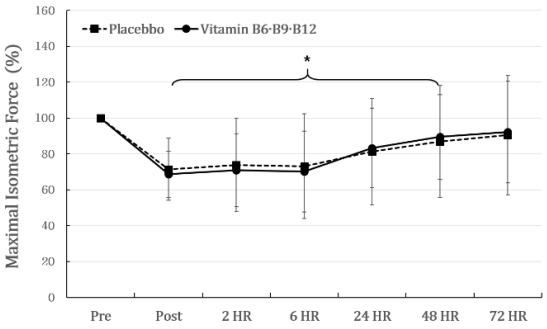


3.3. Muscular function

All subjects performed eccentric exercise to cause muscle damage. During the eccentric exercise, total amount of works were 2213.26 ± 798.56 J and 2349.00 ± 975.41 J for the placebo group and vitamin B6·B9·B12 group, respectively. There was no significant different between groups.

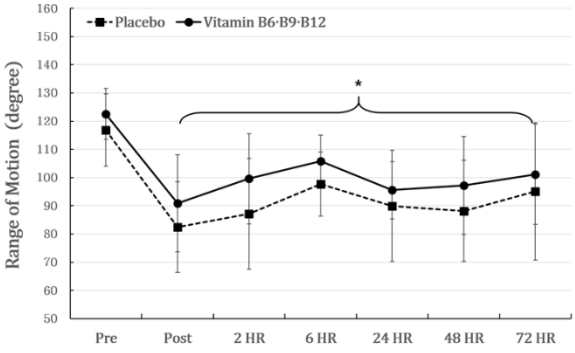
As shown in <Figure 2>, MIF was significantly reduced after eccentric exercise in both groups and demonstrated a time main effect($P < .05$). MIF were declined from immediately after exercise through 48 hours after eccentric exercise compared to pre-exercise in both groups.

Figure 2. MIF changes in response to eccentric exercise over time. Values are presented as mean ± SD. *Significant different compared with Pre (P < .05).



The change of ROM is presented in <Figure 3>. A significant time main effect(P < .05) occurred for the decrease in ROM, independent of treatment from immediately after exercise through 72 hours after eccentric exercise. The reduction of ROM peaked at immediately after exercise. ROM showed to increase after immediately after exercise until 6 hour post-exercise but decreased again after 6 hour post-exercise.

Figure 3. ROM changes in response to eccentric exercise over time. Values are presented as mean ± SD. *Significant different compared with Pre(P < .05).



4. Discussion

The present study examined the effect of a 4-week vitamin B6·B9·B12 supplementation on the muscle recovery and muscular function induced by acute eccentric exercise. As a main findings related to muscle damage, LDH concentration increased gradually until 72 hours after eccentric exercise in both groups, and B vitamins treatment group showed higher LDH concentration compared to the placebo group. As LDH is one of markers of muscle damage, it is an enzyme that exists in the cell. LDH is released from the cell when the cell membrane is damaged by an event such as exercise. In this study, LDH increased up to 72 hours after exercise, and this result means that the eccentric exercise protocol used in this study was enough to induce muscle damage in both groups.

As a main finding of this study, LDH concentration of B vitamins treatment group was higher compared to placebo group. The magnitude of muscle damage could be influenced by various factors including age, sex, skeletal muscle mass, physical activity, and temperature[24]. However, there were no significant differences in total amount of work during eccentric exercise and skeletal muscle mass between two groups in this study.

Another factor that could influence on muscle damage state is the amount of supplements treated. To investigate the effect on muscle damage, Childs et al.(2001)[25] treated vitamin C and N-Acetylcysteine or placebo for 7 days after eccentric exercise. It was demonstrated that the LDH level of vitamin C and N-Acetylcysteine group was significantly higher than placebo group, and they concluded that the increase in LDH might be caused by a chain reaction of oxidation, which is occurred by reaction of supplementations with the increased free irons due to exercise. In addition, Braakhuis, Hopkins, and Lowe(2014)[26] reported that high doses of single vitamin can promote oxidative effects which can exacerbate muscle injury and functions. Pingitore et al.(2015)[27] also suggested that antioxidants may act as pro-oxidant depending on dose, timing, and period of intake. In our study, three subjects showed extremely high concentration of LDH at 72 hour after exercise, and these data were cause of significant group main effect between two groups. However, the average intake rate of these three subjects was 94.79%, and the average intake rate of B vitamin group except the three subjects was 84.38%. Thus, this result indicates that long-term and high levels of vitamin B6·B9·B12 supplementation may have negative effect on muscle damage.

In relation to muscle function, MIF and ROM decreased very fast after acute eccentric exercise, and the patterns of MIF and ROM in the current study are similar with the results of Bryer and Goldfarb(2006)[28]. It is known that the rapid decrease in muscle function occurred immediately after eccentric exercise is influenced by reduction in motor neuron activation, accumulation of lactic acid, depletion of energy sources, and impaired Ca2+ release[29].

Especially, Shafat et al.(2004)[17] and Martin et al.(2004)[30] reported the relationship between the reduction of MIF after eccentric exercise and the excitation-contraction(E-C) coupling failure. E-C coupling is the process that starts with the transmission of action potential from the T-tubule and ends with the cross-bridge of actin and myosin[31]. Also, it was suggested that a problem in the electrical stimulus transportation process and an impaired calcium release during E-C coupling by repeated and high intensity exercise can cause strength loss, and impaired E-C coupling among those factors may have greater effect on the loss of muscle strength compared to muscle damage during 5 days from immediately after eccentric exercise[32]. Therefore, the decrease in MIF observed in this study can be attributed to E-C coupling failure rather than contractile protein damage.

On the other hand, Corona et al.(2010)[33] suggested that contractile protein damage induced after E-C coupling failure, and Hwang et al.(2015)[34] reported that vitamin B9 increased the expression of myoblast differentiation, myotube formation, muscular structure related gene expression. Thus, considering the effects of vitamin B6, 9, and 12 on the synthesis of DNA and protein and the contribution of E-C coupling failure on muscle function damage, it is necessary longer recovery periods over 6 days to examine more accurate MIF recovery by the functioning of vitamin B6·B9·B12 on the recovery of the contractile protein. However, despite the effects of vitamin B6, 9, and 12 on the synthesis of DNA and protein, the intake of these B vitamins during 4 weeks may not seem to protect the strength loss by eccentric exercise induced E-C coupling failure.

In this study, ROM also decreased after eccentric exercise, but ROM started to increase from immediately after exercise until 6 hours after exercise but decreased again at 24 hour post-exercise. The reduction of ROM by muscle damage is related with muscle soreness and muscle circumference. Although these variables were not measured in the current study, it was reported that muscle pain was highest at 24~48 hours after eccentric exercise, and muscle circumference was increased after 48 hours of muscle damage exercise[28][35][36]. Thus, the decrease in ROM after 24 hour post-exercise observed in this study is likely to be the effect of muscle pain and edema. In the case of ROM changes, vitamin B6, 9, and 12 supplementation group showed a tendency of higher ROM compared to placebo group, but statistically, there was no effect of B vitamins supplementation on improving ROM recovery.

5. Conclusion

The present study examined the effects of vitamin B6, 9, and 12 supplementation on the recovery of muscle structural damage and function. It was expected that vitamin B6, 9, and 12 intake would be effective to protect membrane stability and contribute to rapid recovery of muscle strength and ROM by contributing to the recovery of contractile proteins such as myosin and actin. However, vitamin B6, 9, and 12 supplementation for 4 weeks before eccentric exercise did not show protective effect on muscle membrane stability, rather it was found that high dose of B vitamins consumption could have a potential to worsen membrane damage. In addition, a 4-week vitamin B6, 9, and 12 supplementation had no significant impact on the recovery of muscle functional measures. However, it is unclear whether the loss of muscle function measured by MIF and ROM is due to muscular contractile protein damage. In conclusion, we suggest that a long-term supplementation of vitamin B6, 9, and 12 should not be encouraged to prevent muscle damage or to accelerate the recovery from muscle damage due

to the possibility of increasing LDH concentration by functioning as a pro-oxidant.

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