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研究課題名（和文）The potential beneficial effects of inactivated Bifidobacterium Longum on the alleviation of muscle atrophy and physical fatigue in aged hospitalized and athletes using in vitro and in vivo models.

研究課題名（英文）The potential beneficial effects of inactivated Bifidobacterium Longum on the alleviation of muscle atrophy and physical fatigue in aged hospitalized and athletes using in vitro and in vivo models.

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研究成果の概要（和文）：不活化ビフィドバクテリウム ロンガム（IBL）抽出物の抗疲労効果を評価するために、マウスでいくつかの実験を実施しました。 まず、マウスにトレッドミルを使った走行テストを行った。 次に、後肢テストを適用することにより、IBL が不動化に及ぼす影響を調査します。 結果は、非治療マウスと比較して、治療マウスの走行距離が大幅に増加することを示しました。 さらに、結果は、治療グループにおけるパワー強度の顕著な増加を示しました。 さらに、IBL治療グループでは筋肉重量が改善されました。

研究成果の学術的意義や社会的意義
our research showed that IBL can be used for anti-fatigue and muscles atrophy in mice. however, it can be used for human treatment. People who suffering from serious injuries and immobility period due to hospitalization, BL can be a relative treatment.

研究成果の概要（英文）：In order to evaluate the anti-fatigue effect of inactivated Bifidobacterium longum (IBL) extract, some experiments were performed on mice. First of all, mice were exposed to running test using treadmill. Then, we investigate the effect of IBL on immobilization by applying the hindlimb test. The results showed a significant increase of the running distance of treated mice compared with non-treated mice. In addition, the results demonstrated a remarkable increase of power strength in treated groups. Moreover, muscles weight was improved in IBL-treated groups.

研究分野：muscles atrophy and anti-fatigue

キーワード：anti-fatigue　muscles atrophy　treadmill　power strentgh　C2C12 cells

１．研究開始当初の背景

In view of the Increasing interest of community in sports and life quality, furthermore the large number of international sporting events (e.g. Football world cup, Olympic Games ...etc.), appropriate conditions must be associated with such requirements. The biggest obstacle to this majority of people is how to maintain superior fitness and shorten recovery time.

Muscle atrophy, defined as a decrease in the mass and volume of skeletal muscle which is the most abundant tissue in the human body, comprising about 40% of total body mass. Importantly, muscle atrophy is a strong predictor of morbidity and mortality in cardiovascular, musculoskeletal, nervous, renal, and respiratory diseases, as well as cancer. Muscle loss occurs with aging (sarcopenia), reducing functional independence and quality of life as well as increasing the risk of falls and fractures. As in disease states, muscle strength is one of the best predictors of longevity with aging. Therefore, the maintenance of skeletal muscle mass is essential for improving longevity and the quality of life. Moreover, the recovery of athletes during the season aimed to maintain the physical readiness to hold the next competition. Competitive training presents significant mechanical loads and metabolic demands that cause fatigue and represent challenges to the recovery process. Bifidobacterium longum is a commensal bacterium of the human gastrointestinal tract, which has been recognized as safe by the U.S. Food and Drug Administration and by the European Food Safety Authority. Recent studies have shown the anti-allergic, hypocholesterolemia and antiviral effects of BL. The aim of this research is to demonstrate how inactivated Bifidobacterium longum (IBL) has potential beneficial effects on the alleviation of muscles atrophy and physical fatigue.

２．研究の目的

This research will investigate the effects of IBL on muscles atrophy and physical fatigue in in vitro and in vivo. Effect of active Bifidobacterium longum on several biologic activities was investigated. Moreover, few studies were found about the effect of IBL. The application of IBL in foods could offer certain advantages in relation to probiotics, such as: i) minimum interaction with other components of the food products, so extension of shelf life; ii) facility of food processing, as paraprobiotics could be added before thermal processes so as their activity remain to the level required for the intended health benefits; iii) storage and transport simplicity, which could result in longer shelf life and greater convenience for their administration. However, this research will be pioneer study addressed the effect of IBL on muscles atrophy and fatigue in Japan and all over the world. This work will have benefit effects to all categories of society (athletes, hospitalized, aged and hungry people).

３．研究の方法

Competitive training presents significant mechanical loads and metabolic demands that cause fatigue and represent challenges to the recovery process. The aim of this research is to demonstrate how IBL has potential beneficial effects on the alleviation of muscles atrophy and physical fatigue.

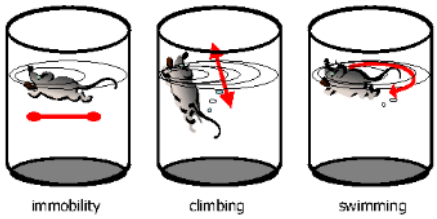
The first part of this study, we investigated the effect of IBL on fatigue in C57BL/6J mice. To induce fatigue, mice were exposed to two different exercises; forced swimming test (FST) and running test (treadmill). Twenty mice were divided into four groups (five mice each); group 1) is the negative control (no treatment and no exercises), group 2) is the positive control (mice were exposed to different exercises without treatment with IBL), group 3) and 4) are 50 mg/kg and 100 mg/kg, respectively (IBL treatment with exercises). After five weeks of treatment, mice were exposed to both exercises with the following order: mice started with FST, then after 30 min, mice were exposed to running test using treadmill. In both exercises, swimming time and running distance were recorded. Thirty min later, power strength of mice muscles (front legs) was measured (MELQUEST, GPM-101B). One hour later, mice were anesthetized by inhalation using isoflurane, then, sacrificed by cervical decapitation. Muscles and livers were collected for further analysis. Muscles were weighing and keeping at -80 C for ATP and glycogen measurements. Moreover, livers were kept at -80° C for glycogen analysis. In addition, blood samples were collected using a heart puncture method, and the vials were kept for 30 min at room temperature, followed by centrifugation to separate serum from the clot. The serum samples were preserved for blood urea nitrogen (BUN) and lactic acid (LA) analysis.

The second part of the project was devoted to investigated the effect of IBL on fatigue and muscles atrophy in C57BL/6J mice and myoblast C2C12 cells. To induce fatigue, mice were exposed to running test (treadmill). I addition, hindlimb was used to induce muscles atrophy. Twenty mice were divided into four groups (five mice each); group 1) is the negative control (no treatment and no exercises), group 2) is the positive control (mice were exposed to hindlimb without treatment with IBL), group 3) and 4) are 10 mg/kg and 50 mg/kg, respectively (IBL treatment with hindlimb). IBL treatment was started 1 week before hindlimb, then continue for 2 other weeks. At the end of treatment (3 weeks), mice were exposed to running test using treadmill. Few mins later, power strength of mice muscles (front legs) was measured (MELQUEST, GPM-101B). One hour later, mice were anesthetized by inhalation using isoflurane, then, sacrificed by cervical decapitation. Muscles and livers were collected for further analysis. Muscles were weighing and keeping at -80 C. Moreover, livers were kept at -80° C for glycogen analysis. In addition, blood samples were collected using a heart puncture method, and the vials were kept for 30 min at room temperature, followed by centrifugation to separate serum from the clot.

In addition, C2C12 cells were used for in vitro studies. The effect of IBL on glucose uptake and some genes expression related to muscles protein degradation (MPD) and muscles protein synthesis (MPS), was investigated. The cytotoxicity effect of IBL was

measured by MTT, and the gene expression was determined by RT-PCR.

Treadmill (for running)



Forced swimming test (for swimming)

This figure shows how to create muscle atrophy by cast immobilization of mouse's hindlimb

４．研究成果

Results showed a significant anti-fatigue effect of IBL in mice. FST results showed a significant increase of mobility time in 50 mg/kg and 100 mg/kg groups to reach 360 s and 440 s, respectively, compared with positive control (200 s). Moreover, the results of running test showed a significant effect of IBL at 50 mg/kg (1100 m) and 100 mg/kg (1300 m) on running distance of mice in dose dependent manner. However, the running distance in positive control group was 600 m. After both exercises, the power strength of mice muscles was measured. Results showed a significant decrease of muscles strength in the non-treated positive control compared with non-fatigue normal control. Furthermore, the power strength of treated mice was significantly increased in dose dependent manner.

BUN is an indicator of how much ammonia (not healthy) is being transferred into urea. Blood serum analysis showed that IBL significantly increased BUN compared with

positive control group. in addition, IBL significantly reduced LA which is the main reason of fatigue feeling. As well, glycogen in muscles and liver was measured. Glycogen is transferred into glucose to produce more energy which can used and help mice for running and swimming. As results, muscles and liver analysis showed a significant decrease of glycogen levels in IBL-treated mice.

The results of second part of work showed a significant anti-fatigue and muscles improvement effect of IBL in mice and C2C12 cells. Moreover, running test showed a significant effect of IBL at 10 mg/kg (450 m) and 50 mg/kg (550 m) on running distance of mice in dose dependent manner. However, the running distance in positive control group was 360 m. After running test, the power strength of mice muscles was measured. Results showed a significant decrease of muscles strength in the non-treated positive control compared with non-hindlimb normal control. Furthermore, the power strength of treated mice was significantly increased in dose dependent manner. Moreover, the Ctrl (+) group showed a significant decrease of muscles weight compared with non-treated Ctrl group. However, at the dose of 50 mg/kg of IBL, the muscles weight was significantly increased compared with Ctrl (+) group. No significant effect was detected of 10 mg/kg of IBL on muscles weight.

The results of MTT showed the safety of IBL even at high dose (500 μg/mL). In addition, IBL significantly increased the glucose uptake at 10, 50 and 100 μg/mL at 6 h to 24 h of treatment. The data of RT-PCR demonstrated an increase of MPS-related genes (Myod-1, mTOR and Myog) and a decrease of MPD-related genes (Myostatin and Atrogin-1).

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関