Aug, 2017

SYNBIO TECH REPORT

Effects of *Lactobacillus plantarum* TWK10 on improvement in endurance during exercise in human
Abstract

*Lactobacillus plantarum* (*L. plantarum*) is a well-known probiotic microorganism, i.e., ingested microorganism associated with beneficial effects in the host. A recent animal study demonstrated that *L. plantarum* TWK10 supplementation could increase muscle mass, improve performance, and exert anti-fatigue effects. Therefore, we conducted a human trial to examine the beneficial effects of TWK10 supplementation on physical fatigue recovery, maximal strength, anaerobic power, endurance performance, and body composition. Sixteen adult subjects aged > 20 years were recruited with at least 8 subjects each randomly allocated to placebo and TWK10 groups, followed by 6 weeks of supplementation. During this experimental period, three time points (pre-, post-, and recovery-test) were applied to evaluate physical fatigue recovery, maximal strength, anaerobic power, endurance performance, and body composition.

I. Research objective

This collaborative study evaluated the effect of *L. plantarum* TWK10, produced by SYNBIO TECH INC., on endurance during exercise in humans according to the “Health food anti-fatigue function evaluation standard” published by the Ministry of Health and Welfare. The anti-fatigue potential of the product is assessed after 6 weeks of continuous intake on the basis of athletic performance and biochemical analysis.

The main objectives of this joint research are as follows:

Objective 1: to examine the role of TWK10 supplementation in improving athletic performance.

Objective 2: to assess the effect of TWK10 supplementation on fatigue-related biochemical indices (blood lactic acid, blood ammonia, blood glucose, and free fatty acid concentrations) and muscle tissue damage (creatine kinase (CK) activity) after exhaustive exercise.

Based on the experimental data and results of these human trials, the evaluation indices that show significant improvement or enhancement are identified as the scientific evidence for the performance of TWK10.

II. Methods and procedures

1. Samples tested
(1) Sample name and composition

*Lactobacillus plantarum* TWK10

(2) Recommended dosage

One bottle contains 45 capsules for the TWK10 group with $1 \times 10^{11}$ CFU/capsule. One capsule is taken per day after breakfast with warm water.

2. Experimental design and procedure

(1) Test subjects

The intended test subjects are 16 healthy male adults between 20–40 years old without professional athletic training.

(2) Test subject grouping

The experiment adopts the double-blind testing procedure, in which the subjects are randomly divided into two groups—placebo and TWK10—with 8 subjects in each group. The test duration was 6 weeks. The blood was immediately drawn after 6 weeks of supplement or placebo intake at the time of exhaustion on the last day of the test.

(3) Diet and physiological activity placebo and management

A. The subjects were asked to maintain a normal diet, and not to consume any nutritional supplements during the test period. They were instructed not to consume alcoholic drinks, yogurt, or probiotic products, such as Yakult, or any drugs during the week before the exercise test and phlebotomy to avoid the influence on experimental results.

B. Subjects were not allowed to consume any food for at least 8 hours before drawing the blood.

C. Subjects were informed to avoid any strenuous physical activities 3 days before the exercise test.

3. Test subjects and experimental methods

(1) Basic physiological examination

Age, height, and weight of the test subjects were recorded.

(2) Maximum oxygen uptake ($V_{O2,max}$) measurement

A. The height and weight of subjects were measured after 8 hours of fasting.

B. Subjects stood on the treadmill (Pulsar, h/p/cosmos, Germany).

C. They wore the Polar heart rate monitoring device during exercise.

D. They also wore respiratory masks before the $V_{O2,max}$ measurement and to ensure there is no gas leak.

E. The measurement was conducted using the Bruse protocol.

F. The SensorMedicsVmax 29c series auto respirato-
ry analyzer was used for gas analysis after gas standardization and gas flow corrections.

G. Gas analyzer was used to analyze the inhaled and exhaled gas of the subjects, and the VO₂ value was calculated. The 3 largest VO₂ values were selected and averaged to find the VO₂max of the subjects. The oxygen uptake was considered maximum when the respiratory exchange ratio (the ratio of carbon dioxide produced to oxygen consumed, VCO₂/VO₂) was above 1.10 and the maximum heart rate was achieved (maximum heart rate = 210–age).

(3) Endurance exercise at 85% VO₂max

A. The subjects should be fasting for more than 8 hours before the test. The blood samples were collected in the morning of the exercise test under fasting conditions in the lab.

B. Before the exercise tests, warm-up was done for 5 minutes at 60% VO₂max, followed by a test on treadmill at 85% VO₂max workload. The heart rate and Borg rating of perceived exertion (Borg RPE) were monitored and recorded until the subjects were exhausted. The sustained exercise duration was recorded.

C. The speed was calculated for 85% VO₂max as workload and 12% as gradient by the ACSM metabolic equivalent equation, which is 85% VO₂max = 3.5 + 0.2 × (speed) + [0.9 × (speed) × 0.12], and is simplified to 85% VO₂max = 3.5 + 0.038 × (speed).

(4) Blood biochemical index analysis

A. Fatigue-related biochemical indices were measured as follows: the maximum oxygen uptake (VO₂max) was measured by drawing blood before exercise. The endurance exercise performance at 85% VO₂max was measured by drawing blood before exercise and at exhaustion. The following 4 fatigue-related biochemical indices—lactic acid, blood ammonia, blood glucose, free fatty acid—and a muscle damage index—CK—were assessed.

4. Statistical analysis

All numerical values are expressed as Mean ± SEM. One-way analysis of variance (ANOVA) was performed using SAS statistical software package. Duncan's test was used to assess the difference between the two groups, with P < 0.05 considered to be statistically significant.

III. Results

The 16 subjects were divided into 2 groups—placebo and TWK10—to achieve balance in basal VO₂max (mL/min/kg). After 6 weeks of continuous supplement intake, the exercise performance and post-exercise fatigue-related blood biochemical indices were measured and analyzed. The results are as shown below.

1. Height, weight, and basal VO₂max for each group

The height of the placebo and TWK10 groups were 176 ± 2 and 173 ± 3 (cm), respectively, as shown in Figure 1A. There was no difference between the two groups (P = 0.3429).

The weight of the placebo and TWK10 groups were 77 ± 5 and 75 ± 5 (kg), respectively, as shown in Figure 1B. There was no difference between the two groups (P = 0.8096).

Based on the maximum oxygen uptake performance, the basal VO₂max of the placebo and TWK10 groups were 44.9 ± 2.4 and 46.2 ± 2.0 (mL/min/kg), respectively, as shown in Figure 1C. There was no difference between the two groups (P = 0.6923).
2. Effects of TWK10 supplementation on endurance exercise

The time to exhaustion was noted to quantify the endurance exercise performance, as shown in Figure 2. After 6 weeks of supplementation, the time to exhaustion for 85% VO2 max workload was 13.6 ± 1.3 and 21.5 ± 3.4 (min) for the placebo and TWK10 groups, respectively, which amounts to 1.58 folds of improvement in the performance of TWK10 group as compared to that of placebo group (P = 0.0475).

3. Effects of TWK10 supplementation on RPE before and after endurance exercise

In addition to the change in heart rate, RPE can be used to evaluate physical activity or exercise intensity in humans, as well as the perceived fatigue after exercise.

As shown in Figure 3, the average RPE for both placebo and TWK10 groups after 3 minutes of exercise (Figure 3A) was both 9 ± 1. There was no significant difference between them (P = 0.6145).

The average RPE for placebo and TWK10 groups at 15 minutes after exhaustion in 85% VO2 max exercise (Figure 3B) were 18 ± 0 and 16 ± 1, respectively. The TWK10 group showed lower RPE (perceived fatigue) than that showed by placebo group, but the difference was not significant (P = 0.2488).

4. Effects of TWK10 supplementation on fatigue-related blood biochemical indices at exhaustion

(1) Effects of TWK10 supplementation on post-exercise blood lactic acid concentration

As shown in Figure 4, the average lactic acid concentration for the placebo and TWK10 groups before exercise (Figure 4A) was 1.9 ± 0.1 and 2.3 ± 0.2 (mmol/L), respectively. No significant difference was
observed between them (P = 0.1556).

The average lactic acid concentration for the placebo and TWK10 groups at exhaustion (Figure 4B) was 13.6 ± 1.3 and 12.3 ± 1.2 (mmol/L), respectively. No significant difference was observed between them (P = 0.4940).

(2) Effects of TWK10 supplementation on post-exercise blood ammonia concentration

As shown in Figure 5, the average blood ammonia concentration for the placebo and TWK10 groups before exercise (Figure 5A) was 54 ± 4 and 52 ± 9 (μmol/L), respectively. No significant difference was observed between them (P = 0.8763).

The average blood ammonia concentration for the placebo and TWK10 groups at exhaustion (Figure 5B) was 137 ± 17 and 134 ± 10 (μmol/L), respectively. No significant difference was observed between them (P = 0.8958).

(3) Effects of TWK10 supplementation on post-exercise blood glucose concentration

As shown in Figure 6, the average blood glucose concentration for the placebo and TWK10 groups before exercise (Figure 6A) was 84 ± 1 and 87 ± 2 (mg/dL), respectively. No significant difference was observed between them (P = 0.1384).

The average blood glucose concentration for the placebo and TWK10 groups at exhaustion (Figure 6B) was 105 ± 9 and 118 ± 6 (mg/dL), respectively. No significant difference was observed between them (P = 0.2534).
4. Effects of TWK10 supplementation on fatigue-related blood biochemical indices at exhaustion

(1) Effects of TWK10 supplementation on post-exercise blood lactic acid concentration

As shown in Figure 4, the average lactic acid concentration for the placebo and TWK10 groups before exercise (Figure 4A) was 1.9 ± 0.1 and 2.3 ± 0.2 (mmol/L), respectively. No significant difference was observed between them (P = 0.1556). The average lactic acid concentration for the placebo and TWK10 groups at exhaustion (Figure 4B) was 13.6 ± 1.3 and 12.3 ± 1.2 (mmol/L), respectively. No significant difference was observed between them (P = 0.4940).

(2) Effects of TWK10 supplementation on post-exercise blood ammonia concentration

As shown in Figure 5, the average blood ammonia concentration for the placebo and TWK10 groups before exercise (Figure 5A) was 54 ± 4 and 52 ± 9 (μmol/L), respectively. No significant difference was observed between them (P = 0.8763). The average blood ammonia concentration for the placebo and TWK10 groups at exhaustion (Figure 5B) was 150 ± 10 and 150 ± 10 (μmol/L), respectively. No significant difference was observed between them (P = 0.8958).

(3) Effects of TWK10 supplementation on post-exercise blood glucose concentration

As shown in Figure 6, the average blood glucose concentration for the placebo and TWK10 groups before exercise (Figure 6A) was 84 ± 1 and 87 ± 2 (mg/dL), respectively. No significant difference was observed between them (P = 0.1384). The average blood glucose concentration for the placebo and TWK10 groups at exhaustion (Figure 6B) was 105 ± 9 and 118 ± 6 (mg/dL), respectively. No significant difference was observed between them (P = 0.2534).

(4) Effects of TWK10 supplementation on post-exercise blood free fatty acid (FFA) concentration

As shown in Figure 7, the average FFA concentration for the placebo and TWK10 groups before exercise (Figure 7A) was 1.02 ± 0.13 and 1.08 ± 0.07 (mmol/L), respectively. No significant difference was observed between them (P = 0.6963). The average blood FFA concentration for the placebo and TWK10 groups at exhaustion (Figure 7B) was 0.62 ± 0.09 and 0.63 ± 0.08 (mmol/L), respectively. No significant difference was observed between them (P = 0.9263).

(5) Effects of TWK10 supplementation on post-exercise muscle damage index—CK

As shown in Figure 8, the average CK activity for the placebo and TWK10 groups before exercise (Figure 8A) was 173 ± 19 and 171 ± 17 (U/L), respectively. No significant difference was observed between them (P = 0.9464). The average CK activity for the placebo and TWK10 groups at exhaustion (Figure 8B) was 277 ± 47 and 256 ± 37 (U/L), respectively. No significant difference was observed between them (P = 0.7402).

IV. Conclusions

From the data of the above pilot study, the effects of TWK10 on exercise performance and blood biochemical indices at exhaustion after six weeks of continuous supplementation were as follows:

1. TWK10 supplementation significantly delayed exhaustion in exercise performance.
2. TWK10 group did not show a difference in the perceived fatigue at exhaustion after exercise, or the blood lactic acid, blood glucose, blood ammonia, and blood free fatty acid concentrations as well as CK activity at exhaustion as compared to those showed by the placebo group.

To sum up, based on the “Health food anti-fatigue function evaluation standard” published by the Ministry of Health and Welfare, *L. plantarum* TWK10 produced by SYNBIO TECH INC. intake of at least 1 serving per day (1 × 10^11 CFU) improves the exercise endurance performance.

V. References

Health food anti-fatigue function evaluation standard

[http://www.fda.gov.tw/TC/siteContent.aspx?sid=1760#.UzxO86I0rSg]
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