PROBIOTIC SUPPLEMENTS FOR ENDURANCE EXERCISE PERFORMANCE AND IMMUNE FUNCTION

SUPLEMENTAÇÃO PROBIÓTICA PARA O DESEMPENHO DO EXERCÍCIO DE RESISTÊNCIA E FUNÇÃO IMUNOLÓGICA

SUPLEMENTACIÓN PROBIÓTICA PARA EL RENDIMIENTO DEL EJERCICIO DE RESISTENCIA Y LA FUNCIÓN INMUNITARIA

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ABSTRACT

Introduction: After long-term research, it was found that athletes are susceptible to suffering from upper respiratory tract infections and digestive system diseases when subjected to high-intensity exercise for long periods. Objective: To verify whether consuming probiotic supplements after exercise can significantly improve the function of the immune system and play a positive role in the health of athletes. Method: This is a quantitative study with distribution analysis to verify whether probiotic supplements could improve immune response after exercise. Results: After using probiotic supplements, by recording the individual differences in the distribution characteristics of athletes› gastrointestinal flora, we found that the changes of subjects› sports performance, leukocytes, neutrophils, lymphocytes, and gastrointestinal flora after six weeks of special training were different. Conclusion: Long-term oral probiotics for athletes can effectively reduce inflammation in the body, reduce damage to the body during exercise, and effectively improve the gastrointestinal tract›s immune function. ***Level of evidence II; Therapeutic studies - investigation of treatment results.***

Keywords: Probiotics; Endurance training; Sports; Immunity; Athletes.

RESUMO

Introdução: Após pesquisas de longo prazo, descobriu-se que os atletas são suscetíveis a infecções do trato respiratório superior e doenças do sistema digestivo quando submetidos a exercícios de alta intensidade por longos períodos de tempo. Objetivo: Verificar se a suplementação probiótica pós-exercício pode melhorar significativamente o funcionamento do sistema imunológico e desempenhar um papel positivo na saúde dos atletas. Métodos: Este foi um estudo quantitativo com análise distributiva para verificar se os suplementos probióticos poderiam melhorar a resposta imunológica após o exercício. Resultados: Após o uso de suplementos probióticos, ao registrar diferenças individuais nas características de distribuição da flora gastrointestinal dos atletas, descobrimos que as mudanças no desempenho esportivo dos sujeitos, leucócitos, neutrófilos, linfócitos e flora gastrointestinal após seis semanas de treinamento especial foram diferentes. Conclusão: Probióticos orais de longo prazo para atletas podem efetivamente reduzir a inflamação no corpo, reduzir os danos corporais durante o exercício e efetivamente melhorar a função imunológica do trato gastrointestinal. ***Nível de evidência II; Estudos terapêuticos – investigação dos resultados do tratamento.***

Descritores: *Probióticos; Treino Aeróbico; Esportes; Imunidade; Atletas.*

RESUMEN

Introducción: Tras una investigación a largo plazo, se descubrió que los atletas son susceptibles de sufrir infecciones del tracto respiratorio superior y enfermedades del sistema digestivo cuando se someten a ejercicios de alta intensidad durante largos períodos. Objetivo: Verificar si el consumo de suplementos probióticos después del ejercicio puede mejorar significativamente la función del sistema inmunológico y desempeñar un papel positivo en la salud de los atletas. Método: Se trata de un estudio cuantitativo con análisis de distribución para verificar si los suplementos probióticos podrían mejorar la respuesta inmunitaria después del ejercicio. Resultados: Después de usar suplementos probióticos, al registrar las diferencias individuales en las características de distribución de la flora gastrointestinal de los atletas, encontramos que los cambios del rendimiento deportivo de los sujetos, los leucocitos, los neutrófilos, los linfocitos y la flora gastrointestinal después de seis semanas de entrenamiento especial eran diferentes. Conclusión: Los probióticos orales a largo plazo para los atletas pueden reducir eficazmente la inflamación en el cuerpo, reducir el daño al cuerpo durante el ejercicio y mejorar eficazmente la función inmune del tracto gastrointestinal. ***Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.***

Descriptores: *Probióticos; Entrenamiento Aeróbico; Deportes; Inmunidad; Atletas.*

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INTRODUCTION

An exercise-induced gastrointestinal syndrome is a gastrointestinal dysfunction caused by athletes during long-term high-intensity, high-volume exercise training and competition. It hinders the athletes' regular sports training and competition to varying degrees. At present, due to the limitation of research methods, the academic community is still inconclusive on the mechanism of the exercise-induced gastrointestinal syndrome.[1] However, scholars have neglected the microflora that colonizes the gastrointestinal tract and is of great significance to the human body in many studies. This is the structure of the gastrointestinal flora. This experiment uses molecular biology methods and techniques to conduct a tentative study on the distribution characteristics of gastrointestinal flora of 7 middle and long-distance runners.

METHOD

Object

This experiment randomly selected 7 health professional athletes (2 males and 5 females) in the long-distance running group as the research objects. All subjects have engaged in professional training for more than 3 years. Good health and no intestinal diseases.[2] The volunteers had not taken antibiotics or other drugs one month before sampling. During the test, we made the subject's diet relatively fixed.

Research methods

Sample processing method

We collect 1 gram of fresh fecal sample under aseptic operation. Suspend the stool sample in 9ml PBS phosphate buffer and vortex until the sample is well mixed. The supernatant was collected after centrifugation at a temperature of 4°C for 5 minutes. This process was repeated 3 times. Then centrifuge at 9000g for 3 minutes and collect the bacteria.[3] Finally, we washed 3 times with PBS phosphate buffer solution and 1 time with sterile water. Then we divided the cells into 1ml/tube.

The extraction method of total bacterial DNA from stool samples

1) We take 1ml of the processed stool sample and shake it vigorously on the vortexes. Mix the sample thoroughly and centrifuge at 90009 for 10 minutes. Pour off the supernatant and resuspend the pellet in 500μl lysis solution I and shake vigorously to mix. 2) We added 100μl of lysozyme (100mg/ml), and the final concentration was 16.8mg/ml. Mix gently and ice bath for 70 minutes. 3) Add 500 μl of lysis solution II and mix well, add aood10% SDS in an ice bath for 5 minutes to lyse the cells, and then divide them into two tubes, each with 620 pl. 4) Add 500μl of Tris-HCl saturated phenol to each tube, mix well, and centrifuge at 1600g for 10min. Then take the supernatant. 5) Use 300 μl of Tris saturated phenol and 300 μl of chloroform isoamyl alcohol with a volume ratio of 24:1 for extraction. Centrifuge at 14000r/min for 15min and take the supernatant. 6) Extract the supernatant with 400μl chloroform. Add 2 volumes of 95% ethanol and precipitate at 20°C for 30 min. 7) Centrifuge at 14000r/min for 20min and remove the supernatant to leave the sediment, and rinse the residue with 1ml of 70% ethanol. 8) After vacuum drying, the residue was dissolved in 20 μl TE. 9) Add 1μl of 20mg/ml RNase and incubate in a water bath at 37°C for 20 minutes.

Making bacterial genetic fingerprints

The primer sequence was designed according to the ERIC sequence reported by Versalovic J. The sequence is

$$(E1\quad 3,\quad CACT-TAGGGGTCCTCCAATGTA\quad 5\quad .$$
$$E2\quad 5)\quad AAGTAAGT-GACTGGGGTGAGGG\quad 3$$

The ERIC-PCR amplification system is 25μl. The amount of primer is 0.5μl (12.5pmol/μl), and the buffer is 2.5μl. The template is 100ng, dNTP 2μl (2.5mM). Taq enzyme is 0.5μl (5U/μl). Makeup 25μl with sterilized ultrapure water.[4] Reaction conditions: pre-denaturation at 95°C for 7 minutes and denaturation at 95°C for 1 minute. Anneal at 52°C for 1 minute. Extend at 65°C for 8 minutes. Cycle 30 times, and finally at 65°C for 16 minutes. The concentration of the PCR product was determined by Dyna quant 200. Each sample was loaded at 400ng/lane and electrophoresed for 60 minutes.

Preparation of probe and nucleic acid blot hybridization

We choose healthy subjects (AH1) with a large amount of information reflected in the genetic fingerprints of intestinal bacteria as the standard.[5] At the same time, we labeled all the PCR products of AH1 with digoxin and used them as probes. Then use this probe to perform blot hybridization with the genetic fingerprints of gastrointestinal bacteria of other subjects (hybridization temperature 68°C).

Molecular cloning

1) Recover and purify the target fragment. 2) Preparation of E. coli DH5 competent cells. 3) Connect and transform the target fragment. 4) Use the alkaline lysis method to prepare a small amount of plasmid DNA. 5) Typing with restriction endonucleases.

Sequencing and comparison of results

1) The cultivated plate is sent to the sequencing company for sequencing. 2) Use Gene. Tool software to sort out the original sequence completed by sequencing. 3) Sequence alignment in GenBank: We COPY the collated sequence in FASTA to BLAST on the NCBI Internet for Nucleotide BLAST and Protein BLAST alignment.

Optimization modeling and simulation of the relationship between sports training and sports injury

P_{wn} represents the type of sports injury. wn represents the nature of the athlete's sports injury. Use the following formula to calculate the relationship between the nature, location, and sports events of sports injuries in different sports in sports training.

$$Q_{(r)}=\frac{y_i y_i \varphi(x_j)}{\alpha_i}\times\frac{K(x,x_i)}{\sin p_{wn}}+b \tag{1}$$

y_i represents the sports injury site of the athlete in sports training. φ represents the general law of sports injuries in different events. x_j represents the athlete's training years. b represents the key joints where athletes are injured during training and $K(x,x_i)$ represents the proportion of the number of people who have suffered injuries during sports training. r represents the cumulative number of injuries of athletes in sports training. Z represents the joints that athletes are vulnerable to injury in sports training. B^* represents the athlete's exercise tolerance in all stages of sports training. q^* represents the athlete's maximum metabolic equivalent before and after sports training.[6] Then use statistics on the parts and types of athletes that are prone to injury in sports training

$$Y_l^*=\frac{[h_k(x_i)\cdot g_l(x_i)]}{T_{Start}\cdot T_{end}}\cdot\frac{\varphi_{crit}}{Q_{(r)}} \tag{2}$$

$h_k(x_i)$ represents the type of factor that caused the damage. $g_l(x_i)$ represents the percentage of factors that cause injuries in sports training. T_{Start} represents various joints that are prone to sports injuries. T_{end} represents the joint part with the highest proportion among vulnerable parts.[7] Use the following formula to get the main factors of athletes' injuries in training

$$f_{nom}(C)=Y_l^*\frac{B^*\cdot Q_{(r)}}{p_{wn}}\times Z_{\Re} \tag{3}$$

$Z_{\Re}$ stands for the detection of sports training intensity. Use the following formula to obtain the regularity formed by the main factors

$$M_{\psi}^{m} = \frac{f_{nom}(C)\beta}{\partial \times \Psi \cdot \Upsilon} \quad (4)$$

β represents the maximum load that the athlete can withstand in the speed and endurance of sports training. ∂ represents the athlete's injury characteristics in sports training. Ψ represents the incidence of sports injuries in athletes' training. Υ represents the nature of sports injuries of athletes in sports training.

RESULTS

Bacterial genetic fingerprinting (ERIC-PCR)

The PCR results (Figure 1) show that each athlete has its unique profile characteristics. The main difference is reflected in the number, location, and brightness of DNA bands. There are 2.4 main bands with higher brightness in each spectrum. This represents some dominant flora.[8] This reflects the individual differences in the distribution characteristics of the gastrointestinal flora of athletes.

Hybridization results

The hybridization results (Figure 2) show that the DNA bands on the genetic fingerprints of the gastrointestinal flora of AH1 and the other 6 athletes have strong hybridization signals. This indicates that the same flora exists to varying degrees among the intestinal flora of different exercise individuals. The results suggest both individual characteristic flora and shared flora among different exercise individuals. In addition, among these hybridization signals, it was found that a signal of about 1.2 kb appeared most frequently. Therefore, it is speculated that the DNA band of about 1.2 kb may be the characteristic band of some bacteria. These bacteria are widely distributed in the intestines of middle and long-distance runners.

Sequencing analysis results

We compare the gene sequences measured by the sequencing company in GenBank. The results showed that the 1.2 kb gene fragment carried in plasmid No. 2 and No. 8 had strong homology with Escherichia coli 0157: H7EDL 933 genome. However, no homologous sequence was found for the 1.2 kb gene fragment carried in plasmid No. 5.

Therefore, from the sequencing analysis results, it can be preliminarily determined that the bacteria represented by the cloned 1.2kb gene fragment belong to Escherichia coli. The specific strain has yet to be further verified. The other type may be a new type of flora that has not been reported yet.

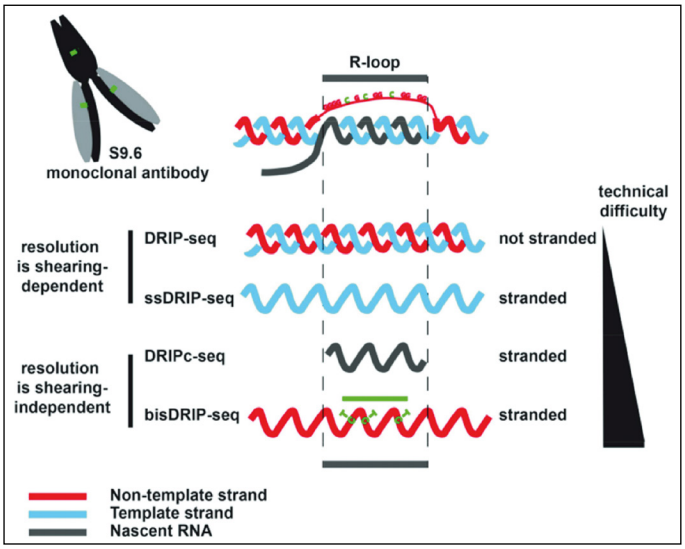

Figure 2. Nucleic acid blot hybridization map.

DISCUSSION

The huge number of microbiotas that colonizes the human gastrointestinal tract contains a wide variety of enzyme systems. It participates in a series of physiological processes such as host energy, material, and genetic information. This has important physiological significance for human health and the normal progress of various life activities. The normal flora of the gastrointestinal tract is an indispensable part of the human body to maintain normal life activities and various physiological stresses. Under normal circumstances, the intestinal flora colonizes the intestinal wall in a certain population ratio and order to form a stable micro ecological balance. Any external factors that can cause changes in the human body's environment can cause changes in the gastrointestinal micro-ecosystem. This study shows that the distribution characteristics of athletes' intestinal flora under the stimulation of the particular stressor of "exercise" show obvious microecological characteristics. Mainly reflected in the distribution structure and number of colonization of the intestinal flora. In addition, we also found that some of the genome fragments representing the common flora of the athlete's gastrointestinal tract are homologous to the gene sequence of E. coli retrieved in the gene bank. In contrast, the other part of the gene sequence that is homologous to it has not been retrieved.

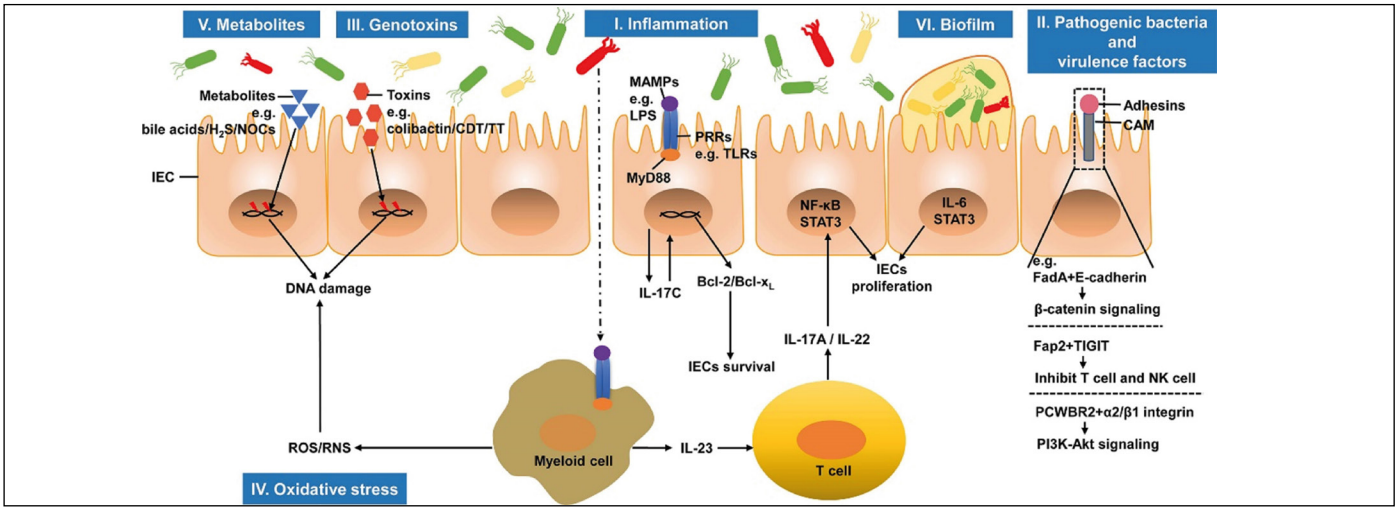

Figure 1. The genetic fingerprints of the gastrointestinal flora of 7 subjects.

On the one hand, there are individual differences in the distribution characteristics of the gastrointestinal flora of the athletes tested and unique similarities. And this common characteristic flora that exists in the intestines of athletes may be a new type of flora that has not been reported. On the other hand, we found that the number of E. coli in the gastrointestinal flora of athletes increased significantly after high-intensity exercise training. As a conditional pathogenic bacterium, Escherichia coli is an important warning factor for changing the intestinal flora structure to the direction that is not conducive to the health of the body. Therefore, the increase in the number of E. coli in the athlete's gastrointestinal tract is likely to destroy the quantitative structure of the gastrointestinal flora, which makes the stability and physiological function of the athlete's gastrointestinal tract potentially dangerous. This is susceptible to harmful external factors. This may be one of the unfavorable factors that cause exercise-induced gastrointestinal dysfunction and intestinal flora imbalance.

The gastrointestinal tract is the largest micro-ecosystem of the human body, and it plays an extremely important role in maintaining the survival and health of the body. This will cause the type and number of normal florae to decrease, or the location of bacterial colonization will change, which will lead to gastrointestinal microecological disorders. Gastrointestinal flora imbalance refers to abnormal changes in normal intestinal flora type, quantity, and proportion. This will lead to a manifestation of adverse effects on the host. However, whether the gastrointestinal motility syndrome causes the gastrointestinal motility syndrome caused by the imbalance of the gastrointestinal flora of the gastrointestinal motility syndrome causes the intestinal flora disorder to be further studied.

CONCLUSION

The distribution structure of the bacteria in the gastrointestinal tract of athletes has both obvious individual differences and common characteristics. A certain characteristic flora in the gastrointestinal tract of middle and long-distance runners does not have a homologous sequence in the gene bank. It may be a new type of flora that has not been reported.

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