Official Title:

Randomized Double-Blind Controlled Trial of Probiotics (Lactiplantibacillus plantarum PD01) for Alleviating the Obesogenic Effect of Microplastics

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Study Protocol and Statistical Analysis Plan

1. Study Background and Rationale

Microplastics (MPs) are ubiquitous in the environment and have been detected in various foods, making oral ingestion a primary exposure route. MPs may induce gut microbiota dysbiosis, activate inflammation, and interfere with energy metabolism, potentially acting as an environmental risk factor for obesity. Probiotics can restore gut homeostasis and enhance barrier function. The strain Lactiplantibacillus plantarum PD01 has shown efficacy in preclinical models in blocking MP translocation and alleviating obesity and inflammation. This study aims to evaluate its efficacy in a human population.

2. Study Objectives

To explore whether probiotic intake can alleviate obesity-related adverse effects caused by microplastic exposure.

3. Study Design

3.1 Study Type

Randomized, double-blind, placebo-controlled intervention trial.

3.2 Participants

Sample Size: 96 healthy volunteers.

Inclusion Criteria:

1. Age 18-65 years.
2. BMI ≥ 24.0 kg/m² OR central obesity (waist ≥ 90cm men, ≥ 85cm women).

Priority given to those with mild blood lipid/liver function abnormalities.

3. Permanent residents of the local area.
4. Voluntary participation and signed informed consent.

Exclusion Criteria:

1. Immunodeficiency, severe allergies, or active gastrointestinal diseases.
2. Use of antibiotics, probiotics, or drugs affecting gut function in the past 6 months.

3. Regular use of nutritional supplements in the past 6 months.
4. Smoking or heavy alcohol consumption.
5. Pregnancy or lactation.
6. Weight change >5% in the past 3 months.

3.3 Interventions

Experimental Group: Probiotic supplement containing 1.8g Fructooligosaccharides (FOS) + 0.2g L. plantarum FABYIO PD01 (10 billion CFU).

Control Group: Placebo containing 2.0g Fructooligosaccharides (FOS).

Dosage: One pack, three times daily, dissolved in warm water (<40°C), taken with/after meals.

Duration: 42 days (6 weeks).

3.4 Randomization and Blinding

Randomization: Participants will be stratified by gender and BMI. A computer-generated randomization list (SAS 9.3) will allocate subjects 1:1 to the two groups.

Blinding: Double-blind design. Both investigators and participants are blinded to group allocation.

3.5 Study Procedures

Visits occur at Baseline (Week 0), Mid-term (Week 3), and End-point (Week 6).

3.6 Outcome Measures

Primary Outcome Measures:

1. Fecal microplastics (MPs) levels: Assessed by Py-GC/MS. Includes detection of 11 types of plastics (PC, PE, PP, PS, PA6, PET, PLA, PVC, PA66, PBAT, PMMA). Reported in μg/g dry weight.
2. Gut microbiota composition: Assessed by Fecal Metagenomics sequencing to analyze diversity and functional genes.
3. Blood metabolic profiles: Assessed by untargeted Blood Metabolomics to identify metabolic pathway alterations.
4. Inflammatory cytokines levels: Measurement of serum markers including MCP-1, IL-1β, IL-6, TNF-α, P-selectin, CRP, etc..

5. Blood biochemical indicators: Assessed by automatic biochemical analyzer. Includes liver function and blood lipid indicators (Total Cholesterol, Triglycerides, HDL, LDL).

6. Routine blood examination: Assessed by automatic blood cell analyzer. Includes white blood cell count (WBC) and five-part differential.

7. Anthropometric and body composition indicators: Measurement of body weight, BMI, waist circumference, hip circumference, body fat percentage, and muscle mass via Bioelectrical Impedance Analysis.

Secondary Outcome Measures:

1. Questionnaire Scores: Assessment of dietary intake (FFQ), physical activity (IPAQ), sleep quality (PSQI), and psychological health (SCL-90).

4. Statistical Analysis Plan

Sample Size: 96 participants.

Analysis Sets: Per-Protocol (PP) set and Intention-to-Treat (ITT) set.

Statistical Methods: Data will be analyzed using SAS 9.3 software. Continuous variables will be presented as Mean ± SD. Repeated measures ANOVA or mixed-effects models will be used to analyze changes in the primary outcome measures (MPs, microbiota, metabolomics, inflammation, biochemistry, blood routine, and InBody data) from baseline to Week 3 and Week 6, comparing the interaction between Group and Time. Significance level is set at $p < 0.05$.

5. Ethics and Safety

The study is approved by the Ethics Committee of the School of Public Health, Fudan University. An adverse event monitoring system is in place.

6. Project Investigators

Principal Investigator: Dong Ruihua

Team Members: Lu Roujun, Zhao Long, Shen Yuyang, Ding Wenbo, Deng Yuyao, Yuan Si.