Version number: V1.1 Version date: 2026.04.23

Tongji Hospital affiliated with Tongji University

# Clinical Nutrition Research Project Proposal

Version Number: V 1.1 | Version Date: 2026.04.23

**Project Title:**Comparison of Short-Peptide vs. Whole-Protein Enteral Nutrition Formulations on Pancreatic Exocrine Function in the ICU

Comparison of protein absorption efficiency among individuals at varying levels of high risk: a multicenter randomized controlled study

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Applicant:Tongji Hospital Affiliated to Tongji University Responsible Department:Critical Care Medicine Center Principal Investigator:Wang Sheng Participating Institution:Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine;

The Fourth People's Hospital Affiliated to Tongji University

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## Researchers' Statement and Protocol Signing Page

As the principal investigator of this research project, I will adhere to the ethical review guidelines established by the Ministry of Health (Measures for the Ethical Review of Biomedical Research Involving Human Subjects, 2016), the WMA Helsinki Declaration (2013), the CIOMS International Ethical Guidelines for Biomedical Research in Humans (2002), and the ethical principles of Good Clinical Practice (GCP). Under the guidance of Good Clinical Practice standards, I will implement the protocol approved by the ethics committee and conduct the study in accordance with its requirements to ensure scientific rigor while safeguarding the health and rights of participants.

surname and personal name ： _____

sign one's name ： _____

date ： _____

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## Scenario Summary

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| Plan Title | Comparison of protein absorption efficiency between short-peptide and whole-protein enteral nutrition formulations in high-risk populations with pancreatic exocrine insufficiency (PEI) in the ICU: a multicenter randomized controlled study |
| Version Number/ Version Date | V1.1/2026.04.23 |
| Applying and Participating Organizations | Applicant: Tongji Hospital Affiliated to Tongji University<br>Participating institution: Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine;<br>The Fourth People's Hospital Affiliated to Tongji University |
| Principal Investigator | Wang Sheng |
| Nature of the Study | Intervention study |
| Purpose of research | **Main research objective**: Using targeted amino acid metabolomics and gut microbiota co-metabolomics as core quantitative indicators, this study systematically evaluates whether short-peptide-based versus whole-protein-based enteral nutrition (EN) formulations can enhance protein absorption efficiency in high-risk patients with post-enteral nutrition (PEI) on the intensive care unit (ICU).<br>**Secondary research objectives**: To compare the effects of two EN formulations on pancreatic exocrine function, gastrointestinal tolerability, associations with intestinal barrier function and gut microbiota metabolic profiles, as well as clinical outcomes in study subjects. |
| Sample Size | 250 Intervention group: Control group = 1:1 |
| Subject investigated | Intestinal nutrition should be administered to critically ill patients in the ICU who have high-risk factors for PEI. |
| Research technique | Multicenter randomized controlled study |
| Inclusion Criteria | 1. Age ≥18 years; no gender restriction.<br>2. Meets the criteria for the high-risk population under PEI;<br>3. AGI ≤ grade II, hemodynamic stability, meeting the criteria for EN initiation;<br>4. The duration of enteral nutrition support is expected to be ≥7 days.<br>5. The study subject or their legally authorized family member signs the informed consent form. |
| Exclusion Criteria | 1. Patients with a definitive diagnosis of PEI or chronic persistent PEI;<br>2. Patients with contraindications to enteral nutrition therapy;<br>3. Patients with severe impairment of hepatic and renal function; |

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| | 4. Patients with combined amino acid metabolism disorders, severe malnutrition, or cachectic state;<br>5. Had received pancreatic enzyme replacement therapy or had conditions that might affect protein metabolism within 7 days prior to enrollment. |

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| | Patients undergoing drug metabolism enhancement therapy;<br>6. The expected ICU stay is less than 7 days.<br>7. Women who are pregnant or breastfeeding, or patients with comorbid mental disorders;<br>8. Allergic to any component of the investigational preparation. |
| End-of-Experiment Criteria | 1. completion of the final planned procedure specified in the study protocol shall be deemed as the subject having completed the study.<br>2. The study is considered concluded upon the completion of the final visit for the last participant. |
| Deletion/Exclusion Criteria | 1. After enrollment, individuals who did not meet the inclusion criteria or exhibited any of the exclusion criteria were identified.<br>2. After enrollment, for various reasons, the duration of enteral nutrition support was less than 7 days, making it impossible to complete the full intervention cycle.<br>3. Key data are missing due to difficulties in specimen collection, inability to obtain qualified test specimens, or invalid test results.<br>4. The study subject or their family members voluntarily requested to withdraw from the study;<br>5. The patient was lost to follow-up during the follow-up period, making it impossible to collect outcome data. |
| Early Exit Criteria | 1. When a subject experiences a serious adverse event and/or severe complication, the investigator determines that discontinuation of participation in the study is warranted for the benefit of the subject.<br>2. The patient's AGI score has progressed to AGI III or higher, and/or the tolerability score is ≥5.<br>3. The researchers concluded that, in consideration of the subjects' interests, discontinuing the study was the optimal choice.<br>4. Clinical assessment and confirmation by the research team indicated that pancreatic enzyme replacement therapy or switching to an alternative enteral nutrition formulation was required due to the patient's condition. |
| Dosage regimen | • During routine assessment, when the patient is deemed eligible for initiation of enteral nutrition, continuous infusion is administered via a nasogastric tube or nasoenteric tube at an initial rate of 10–20 mL/h, with the infusion rate gradually adjusted based on tolerance.<br>• Target Quantity:<br>o D 1–2: ≥250 kcal/day<br>o D3 and later: ≥500 kcal/day<br>o Refer to the energy target of 25–30 kcal/kg/day and the protein target. |

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| | 1.2−1.5 g/kg/day, with the target feeding dose determined based on individual patient conditions<br>o Oral feeding should be initiated slowly and gradually increased in speed, with the infusion rate adjusted according to the patient's tolerance until the target rate is achieved.<br>▪ D 1: Initial infusion rate ≤ 20 mL/h<br>▪ D2: Initial drip rate ≤ 40 mL/h<br>▪ D3: Initial drip rate ≤ 60 mL/h |

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| | ▪ For D4 and later: Initial infusion rate ≤60 mL/h. Record the patient's daily dietary intake and protein consumption.<br>• Duration: ≥7 days, but the study was terminated after 14 days.<br>**Experimental group:**Short-peptide type EN preparation<br>**Control group:**Whole-protein EN formulation |
| Main efficacy indicators | The core indicator assessing its impact on protein absorption efficiency in high-risk populations for PEI is: 1. Plasma branched-chain amino acid (BCAA) AUC −4 h;<br>2. Plasma citrulline concentration;<br>3. Urine 3-methylhistidine concentration;<br>4. 24-hour nitrogen balance value. |
| Secondary efficacy endpoints | 1. Pancreatic function indicators: fecal elastase-1, serum pancreatic lipase, and amylase;<br>2. Intestinal function and microbiota parameters: plasma DAO and D-lactate levels, fecal p-cresol and indole content, etc.;<br>3. Clinical outcome indicators: mechanical ventilation duration, ICU hospitalization duration, total hospitalization duration, incidence of infection complications, degree of rectus femoris muscle atrophy on day 14 post-intervention, and 28-day all-cause mortality rate.<br>4. Feeding tolerance indicators: incidence and duration of diarrhea, abdominal distension, and gastric retention. |
| Safety Indicator | The incidence rates of adverse reactions related to enteral nutrition, allergic reactions, and abnormalities in liver and kidney function parameters. |
| Research Progress Plan | 2026.07.01—2027.12.31 |
| Statistical Analysis Methods | All statistical analyses were performed using SPSS 26.0 and R 4.4.0 statistical software. The study strictly adhered to the STROBE guidelines, with all statistical tests conducted as two-sided tests; a $P<0.05$ was considered statistically significant.<br>Continuous variables were described as mean ± standard deviation or median (quartiles) depending on their distribution type; categorical variables were expressed as frequency (percentage). For normally distributed and homoskedastic quantitative data, the t-test was employed; otherwise, the non-parametric test was used. Inter-group comparisons were performed using analysis of variance or the Kruskal-Wallis H-test. Categorical data were analyzed with the $\chi^2$ test or Fisher's exact probability method. Multivariate linear regression was conducted to evaluate the effect of formulation type on protein absorption efficiency; logistic regression was used to analyze the association between protein absorption efficiency and clinical outcomes; Cox proportional hazards regression was employed to examine the relationships |

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| | among formulation type, protein absorption efficiency, and 28-day all-cause mortality. |
| Form of publication for research findings | 2 academic papers |

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## II.Project Research Content (Please do not delete the explanatory text in the table)

**(1) Research Background and Basis, Research Objectives, Study Design, Main Research Content, Research Methods and Implementation Plan (**including study population (inclusion/exclusion/exit criteria), sample size estimation, intervention protocol, end-point indicators, follow-up schedule, etc.)**, Statistical Analysis Methods**

## 1. Research Background and Basis

In the ICU, critically ill patients often face a dual challenge of hypermetabolism and impaired protein absorption due to severe stress states, which interact to create a vicious cycle. This leads to rapid skeletal muscle atrophy, delayed wound healing, significantly increased risks of infectious complications, prolonged mechanical ventilation duration, and extended ICU hospitalization time, severely compromising patient recovery and long-term prognosis—a critical issue that demands urgent resolution in critical care medicine [1–3]. Our previous epidemiological data indicate that the incidence of pancreatic exocrine insufficiency (PEI) among ICU patients reaches as high as 52.2%, with severe PEI accounting for 18.3%. Shock, sepsis, cardiac arrest, hyperlactatemia, and invasive mechanical ventilation are identified as high-risk factors for PEI in the ICU setting [5]. PEI refers to dysfunction of the pancreatic exocrine glands, resulting in insufficient secretion of digestive enzymes and subsequent impairment of digestion and absorption processes [4]; thus, individuals at high risk for PEI represent a subpopulation with a heightened susceptibility to protein absorption disorders. Moreover, administering conventional nutritional formulations to critically ill patients with PEI not only fails to correct malnutrition but may also exacerbate gastrointestinal burden, inducing adverse effects such as abdominal distension, diarrhea, and pancreatic enzyme secretion disturbances, thereby further deteriorating patient outcomes [6–7]. Therefore, in critically ill patients exhibiting both the dual pathophysiological characteristics of "ICU stress state + high risk for PEI," implementing enteral nutrition therapy is more challenging, and the risk of poor prognosis is significantly higher.

**Enteral nutrition (EN), as a nutritional support approach that aligns with human physiological digestion and absorption mechanisms, has been explicitly recommended by multiple clinical guidelines as the preferred method for nutritional support in ICU patients. Among these, short-peptide formulations and whole-protein formulations are currently the two most widely used EN options in clinical practice [8–9]. Short-peptide formulations primarily utilize small-molecule peptides as their protein source, requiring no extensive enzymatic digestion; they can be directly absorbed via the PepT1 transporter on intestinal epithelial cells, making them theoretically more suitable for patients with pancreatic enzyme deficiency or impaired digestive function [10]. Whole-protein formulations, on the other hand, rely on intact proteins as their core component, necessitating digestion by pancreatic enzymes such as trypsin and chymotrypsin for absorption. While offering advantages including low cost, comprehensive nutritional profile, and broad applicability, their absorption efficiency may significantly decrease in patients with pancreatic exocrine insufficiency (PEI), failing to meet nutritional demands [11–12]. However, current clinical practice for selecting EN formulations in high-risk PEI patients predominantly depends on clinicians' empirical judgment rather than robust evidence-based evidence.**Moreover, previous related studies exhibit significant limitations: first, the study populations predominantly focused on conventional ICU patients or patients with chronic protein malabsorption (PEI) (e.g., postoperative patients with chronic pancreatitis or pancreatic cancer), failing to specifically address the dual pathophysiological characteristics of "ICU stress state + high risk of PEI," resulting in poor clinical generalizability of the findings [13–15]; second, most studies were single-center, small-sample retrospective observational studies with low evidence quality, and core evaluation metrics primarily centered on feeding tolerance without achieving precise quantitative assessment of protein absorption efficiency [16–17]; third, evaluation methods were limited to macroscopic parameters such as nitrogen balance and plasma total amino acid concentrations, which could not effectively distinguish the impacts of different pathological mechanisms—including insufficient pancreatic enzyme digestion, intestinal absorption disorders, and hypercatabolism of muscle tissue—on protein metabolism, thereby hindering identification of the underlying pathophysiological basis for differences in absorption between the two formulations [18–19].

The 2025 Nutrition Guidelines for Severe Patients issued by the American Society for Parenteral and Enteral Nutrition (ASPEN) explicitly state that targeted nutritional intervention studies should be conducted for populations with specific pathological conditions in the ICU (e.g., high-risk groups for parenteral enteral nutrition [PEI], patients with acute gastrointestinal injury, etc.). The guidelines prioritize the use of enteral nutrition (EN) formulations tailored to their digestive and absorptive capabilities to enhance the clinical efficacy of nutritional support therapy and improve patient outcomes [20].

In recent years, the rapid advancement of targeted amino acid metabolomics has provided novel technical support for the precise quantification of protein absorption efficiency. This technology enables systematic evaluation of the entire protein processing pathway—from digestion to absorption and metabolism—by accurately detecting concentration changes of specific metabolic markers such as branched-chain amino acids (BCAAs) and citrulline in plasma, combined with analysis of fecal microbiota co-metabolites, thereby overcoming the limitations of traditional assessment methods [21–23]. Addressing these clinical challenges, existing research gaps, and technological constraints, this study focuses on high-risk populations with pancreatic exocrine insufficiency in the ICU. Using whole-protein enteral nutrition formulations as controls, a multicenter prospective randomized controlled trial will systematically evaluate the effects of short-peptide enteral nutrition formulations on protein absorption efficiency, pancreatic exocrine function, gastrointestinal tolerance, interactions with intestinal barrier function and gut microbiota metabolic profiles, as well as clinical outcomes in these high-risk groups. The findings aim to provide high-quality evidence-based data for developing personalized EN formulation selection strategies and promote the transition of critical care nutrition therapy from an empirical approach to a precision-based one.

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## 2. Purpose of Research

2.1 Main Research Objectives:

A multidimensional evaluation system was established, utilizing targeted amino acid metabolomics (plasma branched-chain amino acid area under the curve, plasma citrulline concentration, and urinary 3-methylhistidine concentration) and gut microbiota co-metabolomics (fecal p-cresol and indole content) as core quantitative indicators, combined with traditional assessment metrics (24-hour nitrogen balance), to precisely quantify differences in protein absorption efficiency between short-peptide and whole-protein EN formulations among high-risk patients with post-enteral nutrition (PEI) in the intensive care unit (ICU).

2.2 Secondary research objectives:

2.2.1 Analyze the effects of two formulations on the pancreatic exocrine functions (Levels of fecal elastase-1, serum pancreatic lipase, and amylase) in study subjects; validate the potential impacts of different formulations on pancreatic function; and assess the safety of short-peptide formulations in high-risk populations for PEI.

2.2.2 During the intervention period, the incidence and severity of gastrointestinal adverse reactions (e. g., vomiting, diarrhea, abdominal distension, gastric retention) were compared between the two formulations to evaluate the gastrointestinal tolerability of the short-peptide formulation.

2.2.3 Analysis of two EN formulations and their effects on intestinal barrier function (plasma diamine oxidase [DAO], plasma D-lactate), as well as gut microbiota composition.

To investigate the correlation between fecal p-cresol and indole content, and to explore the mechanisms by which formulation types affect gut microbiota and intestinal barrier integrity.

2.2.4 The study evaluated differences in clinical outcomes between the two EN formulations after intervention, including core clinical indicators such as mechanical ventilation duration, ICU hospitalization duration, total hospital stay duration, incidence of nosocomial infections (ventilator-associated pneumonia, catheter-related urinary tract infection, catheter-related bloodstream infection), rectus femoris muscle atrophy severity on day 14 post-intervention, and all-cause mortality at day 28.

## 3. Research Design

3.1 Research Type

This study adopted a multicenter randomized controlled trial design, with participants randomly assigned to groups without mandatory intervention measures. The study was conducted strictly in accordance with standard clinical practice guidelines to ensure the real-world applicability of the results.

3.2 research center

The study plans to include intensive care units (ICUs) from 3 to 5 domestic tertiary Grade A hospitals. All participating centers must meet the following criteria: possess standardized diagnostic and therapeutic workflows for enteral nutrition support along with standardized operating procedures (SOPs); have the necessary hardware facilities and technical capabilities for plasma amino acid testing, pancreatic enzyme level measurement, and metabolomic sample storage; and maintain experienced ICU clinical and research teams capable of ensuring smooth study implementation and data quality.

3.3 Grouping Method

Study subjects meeting the inclusion criteria were randomly assigned in a 1:1 ratio to either the short peptide group or the whole protein group according to the specified allocation protocol, as detailed below:

1）Short peptide group: Administer a short-peptide-enriched EN formulation for nutritional support therapy. The selected formulation must have a protein content of 5 g/100 ml, consist entirely of hydrolyzed whey protein, and be readily absorbed by the intestines without requiring full pancreatic enzyme digestion.

2）Whole Protein Portfolio: Administer whole protein-enriched EN formulations for nutritional support therapy, with selected formulations requiring a protein content of 5 g/100 ml.

They are all complete protein components that are digested and absorbed after being broken down by pancreatic enzymes secreted by the pancreas.

Both study groups strictly adhered to the 2025 ASPEN Guidelines for Nutritional Therapy in Critically Ill Patients and the expert consensus on critical care nutrition in China when administering nutritional support therapy. Apart from differences in enteral nutrition (EN) formulations, all other therapeutic interventions—including anti-infective therapy, organ function support therapy, gastrointestinal motility regulation therapy, and sedation/analgesia—were rigorously implemented in accordance with standard ICU clinical protocols, ensuring consistent baseline treatment conditions between the two groups.

3.4 Blind design

Due to significant differences between short-peptide and full-protein EN formulations in terms of dosage form (suspension/emulsion), taste profile, nutritional composition ratio, and clinical application scenarios, it is challenging to implement a double-blind design involving both study subjects and healthcare professionals. To minimize the impact of assessment bias on research outcomes, this study adopted a single-blind design for outcome evaluators: neither the laboratory technicians performing parameter measurements nor the statistical analysts were aware of participant assignments. Sample management and data processing employed coding methods throughout the study, with group assignments being decoded only after all data had been finalized.

3.5 Research Period

The total study duration was set at 18 months, divided into two phases: the first phase (enrollment period) lasted 16 months, during which all subjects underwent screening, enrollment, and intervention implementation; the second phase (follow-up period) lasted 2 months, ensuring that all enrolled subjects completed follow-up visits 28 days after intervention completion and enabling comprehensive collection of study outcome data.

## 4. Main Research Content

- **Comparative Study on Protein Absorption Efficiency**: A multidimensional evaluation system integrating "targeted amino acid metabolomics markers + traditional assessment indicators + pancreatic function parameters" was established. By precisely measuring metabolic biomarkers such as plasma branched-chain amino acids, plasma citrulline, and urinary 3-methylhistidine, combined with nitrogen balance data, plasma total amino acid concentrations, and pancreatic enzyme levels, this approach systematically quantified the differences in protein absorption efficiency between two enteral nutrition (EN) formulations among high-risk patients undergoing parenteral enteral nutrition (PEI) in the intensive care unit (ICU).
- **Analysis of Protein Absorption Differences Mechanisms**: Correlation analyses were conducted between pancreatic function indicators (fecal elastase-1, serum pancreatic enzyme levels), intestinal barrier function indicators (plasma DAO, D-lactate), gut microbiota metabolic profiles (fecal p-cresol, indole), and protein absorption efficiency parameters, systematically elucidating the core mechanism by which short-peptide EN formulations are tailored for high-risk populations undergoing ICU PEI.
- **Clinical outcome association analysis**: Through follow-up observation, this study investigated whether higher protein absorption efficiency could effectively reduce muscle wasting and the risk of infectious complications in high-risk ICU patients with PEI, thereby improving short-term clinical outcomes (mechanical ventilation duration, hospital stay) and prognosis (28-day all-cause mortality).
- **Gastrointestinal tolerance assessment**: Detailed records were maintained of gastrointestinal adverse reactions (vomiting, diarrhea, abdominal distension, gastric retention) in both patient groups during the intervention period.

The occurrence time, frequency, severity, and clinical intervention measures of adverse reactions (including retention and reflux). The total incidence rate of adverse reactions and the proportion distribution by severity levels were calculated for both groups, and differences in gastrointestinal tolerability between the two formulations were compared.

- **Evaluation of pancreatic exocrine function**: Dynamic monitoring of pancreatic exocrine function-related parameters—including serum amylase, lipase, and fecal elastase-1 (FE-1) levels—was performed in both groups before intervention and at 1 week post-intervention. When necessary, pancreatic ultrasound or CT scans were employed to assess morphological changes in the pancreas.

# 5. Research Methods and Implementation Plan

## 5.1 Study Population

### 5.1.1 Selection Criteria

- Patients aged ≥18 years, regardless of gender, who meet the temporal requirements for baseline data collection and intervention implementation.
- Meets the definition of high-risk populations for PEI and satisfies at least one of the following criteria: APACHE II score ≥15, indicating severe disease status, significant stress response, and elevated risk of pancreatic dysfunction; Postoperative period ≤7 days following major abdominal surgery (including gastrointestinal, hepatobiliary, or pancreatic surgeries), during which postoperative stress may readily impair pancreatic function; Concurrent severe acute pancreatitis meeting diagnostic criteria in the Atlanta Classification (2012 edition); Diagnosis of sepsis (SOFA score ≥2) accompanied by elevated serum pancreatic lipase/ starchase levels (>1.5 times upper limit of normal), suggesting pancreatic involvement; Prolonged fasting (≥72 hours) with concomitant gastrointestinal dysfunction (gastric retention> 200 mL/4 h or diminished/absent bowel sounds), indicating impaired digestive and absorptive functions.
- Hemodynamic stability (maintained with norepinephrine at a dose of ≤0.2 μg/kg/min or equivalent doses of other vasoactive agents; blood lactate concentration ≤2 mmol/L; urine output ≥0.5 ml/kg/h) and an AGI score ≤ grade II meet the criteria for initiating enteral nutrition therapy.
- The duration of enteral nutrition support is expected to be ≥7 days, with feeding administered via nasogastric tube or nasoenteric tube, ensuring continuous implementation of intervention measures and effective monitoring of outcome indicators.
- The study subject or their legally authorized family member signs the informed consent form.

### 5.1.2 Exclusion Criteria

- Patients with a definitive diagnosis of PEI (fecal elastase-1 <200 μg/g) or those with concurrent chronic pancreatic diseases (including chronic pancreatitis, pancreatic cancer, etc.) exhibit chronic and persistent pancreatic dysfunction, which contradicts the high-risk population for acute stress-induced PEI focused on in this study.
- There are contraindications to enteral nutrition therapy, including paralytic ileus, active upper gastrointestinal bleeding, short bowel syndrome, severe intestinal ischemia, etc., making enteral nutritional support impossible.
- Concomitant severe hepatic or renal dysfunction (Child-Pugh liver function score ≥ grade C) and stage 3 acute kidney injury (AKI) without

In cases of repeated RRT (renal replacement therapy) or prolonged RRT duration exceeding 7 days, severe impairment of hepatic and renal function may affect protein metabolism and the accuracy of test parameters.

- Patients with combined amino acid metabolic disorders (e.g., maple syrup urine disease), severe malnutrition (BMI <16 kg/m²), or cachectic state exhibit congenital or severe pathological abnormalities in protein metabolism, which may interfere with study outcomes.
- Patients who received pancreatic enzyme replacement therapy (PERT) or medications potentially affecting protein absorption (e.g., long-term use of glucocorticoids for>1 week at doses exceeding 20 mg/day equivalent to prednisone) within 7 days prior to enrollment may have had their pancreatic function and protein absorption efficiency assessed compromised.
- Patients with an anticipated ICU stay of <7 days, those scheduled for transfer to another medical institution, or those whose family members have explicitly declined active treatment are unlikely to complete the full intervention and follow-up process.
- Women who are pregnant or breastfeeding, those with mental disorders who cannot cooperate with the study investigators, individuals at risk of ethical issues in the study, or cases where the study implementation poses significant challenges.
- Participated in other interventional clinical studies during the same period.

#### 5.1.3 Exit Standards

- After enrollment, individuals who did not meet the inclusion criteria or exhibited any of the exclusion criteria were identified.
- After enrollment, the duration of enteral nutrition support was less than 7 days, including cases where transition to total parenteral nutrition due to disease progression, subject death, transfer to a non-partnering research center, or inability to complete the full intervention cycle.
- The occurrence of severe adverse reactions or complications (such as severe diarrhea, intense vomiting, etc.) that remain intolerable to enteral nutrition therapy despite symptomatic management necessitates termination of the intervention.
- Allergy to any component of the investigational preparation;
- During the study period, initiation of pancreatic enzyme replacement therapy or adjustment of the enteral nutrition (EN) formulation type due to clinical needs (not required by the study protocol) may interfere with the evaluation of study parameters.
- The absence of key data prevents the conduct of primary outcome analysis due to difficulties in specimen collection, inability to obtain qualified test specimens, or invalid test results.
- The study subject or their family members voluntarily requested to withdraw from the study;
- The patient was lost to follow-up during the follow-up period, making it impossible to collect outcome data.

### 5.2 Sample Size Estimation

This study estimated the sample size based primarily on the key outcome endpoint, "plasma BCAA area under the curve (AUC −4 h)." Referring to results from relevant domestic and international research literature, the plasma BCAA AUC −4 h of short-peptide EN formulations in critically ill patients was higher than that of other formulations.

The whole-protein EN formulation showed an approximately 25% increase. Based on this, the following research hypothesis was established: the mean plasma BCAA AUC −4 h value in the whole-protein group was 1200 μmol·h/L with a standard deviation of 300 μmol·h/L; whereas in the short-peptide group, the mean value was 1500 μmol·h/L with a standard deviation of 320 μmol·h/L, and the difference between the two groups was clinically significant.

The intergroup differences were compared using a two-sample t-test with an alpha level of $\alpha$ = 0.05 (two-sided test) and power of $1 - \beta = 0.80$ (equivalent to $\beta = 0.20$). Sample size calculations were performed using the PASS 15.0 statistical software, yielding a required sample size of 102 cases per group. Considering the potential 20% dropout rate in multicenter studies (including follow-up discontinuations and data missing values), the total study sample size needed was 245 cases to ensure statistical power; consequently, 250 participants were planned (approximately 125 cases per group) to guarantee reliable and robust results. In accordance with the multicenter design involving three hospitals, each center was required to enroll at least 80 cases (approximately 40 cases per group);our center plans to enroll 100 cases.

### 5.3 Intervention Strategy

#### 5.3.1 Nutritional Support Objectives

Nutritional support targets strictly adhere to the 2025 ASPEN Guidelines for Nutritional Therapy in Critically Ill Patients: The energy target is 25–30 kcal/kg/day (calculated based on the patient's actual body weight), with indirect calorimetry (IC) being the preferred method for determining resting energy expenditure (REE); energy intake should be precisely tailored according to these measurements. For research centers lacking IC facilities, the Penn State 2010 formula is used to estimate resting energy expenditure. The protein target ranges from 1.2 to 1.5 g/kg/day (calculated based on the patient's actual body weight), ensuring adequate protein intake to meet the demands of a hypermetabolic state in critically ill patients.

#### 5.3.2 Selection of Medication and Infusion Method

5.3.2.1 Short peptide group: Select a short-peptide EN formulation (protein content 5 g/100 ml, composed entirely of hydrolyzed whey protein);

5.3.2.2 Whole Protein Preparation: Select the whole protein type EN formulation (protein content: 5 g/ 100 ml; 100% complete protein).

5.3.2.3 Administration method: Continuous infusion is performed via nasogastric tube or nasoenteric tube, with an initial infusion rate of 10–20 mL/h. Throughout the infusion process, closely monitor the patient's feeding tolerance (for symptoms such as diarrhea, abdominal distension, or gastric retention). Gradually increase the infusion rate by 20–30 mL/h daily based on tolerance, with the ultimate target rate being 40–60 mL/h. Maintain a head-of-bed elevation of 30–45° during infusion to effectively prevent aspiration complications. Regularly assess gastric retention volume and adjust the infusion regimen accordingly.

#### 5.3.3 Basic Treatment Protocol

All basic therapeutic measures, including anti-infective therapy, organ function support therapy (including respiratory support, circulatory support, renal function support, etc.), gastrointestinal motility regulation therapy (such as the use of mosapride or erythromycin), and sedation/analgesia therapy, were strictly implemented in both study groups.

The procedures shall be carried out in accordance with the standard ICU diagnostic and treatment protocols as well as relevant disease-specific guidelines. During the study period, medications that may impair protein absorption (e.g., pancreatic enzyme preparations, high-dose glucocorticoids) should be avoided whenever possible. If such medications are indeed required due to the patient's condition, detailed records must be maintained regarding dosage, duration of administration, and rationale for use, to serve as adjustment factors for subsequent statistical analysis.

### 5.4 Endpoint Criteria

#### 5.4.1 Primary End Point Indicator

The rapid advancement of targeted amino acid metabolomics technologies has provided novel technical support for the precise quantification of protein absorption efficiency. This approach enables systematic evaluation of the entire protein processing pathway—from digestion to absorption and metabolism—by accurately detecting concentration changes of specific metabolic markers such as branched-chain amino acids (BCAAs) and citrulline in plasma, combined with analysis of fecal microbiota co-metabolites, thereby overcoming the limitations of traditional assessment methods.

Protein absorption efficiency: The core quantitative parameter is the plasma BCAA (including leucine, isoleucine, and valine) AUC −4 h on day 7 of intervention. The specific measurement protocol involves collecting venous blood samples from patients in a fasting state, followed by initiating enteral nutrition infusion. Venous blood samples are collected at 0.5, 1, 2, 3, and 4 hours post-infusion to measure plasma BCAA concentrations at each time point and calculate the AUC −4 h. Comprehensive evaluation is performed by combining plasma citrulline concentration (reflecting intestinal epithelial absorption function), urinary 3-methylhistidine concentration (indicating muscle protein breakdown), and 24-hour nitrogen balance values. Detection methods: Plasma amino acid levels, plasma citrulline concentration, and urinary 3-methylhistidine concentration were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Nitrogen balance was calculated as: Nitrogen balance = Intaken nitrogen (protein content of EN formulations / 6.25) − (Fecal nitrogen + Urinary nitrogen + Skin nitrogen loss), with skin nitrogen loss estimated at 2 g nitrogen per day.

#### 5.4.2 Secondary Endpoint Indicators

5.4.2.1 Pancreatic function indicators

Samples were collected at enrollment and on day 7 of intervention for testing, including fecal elastase-1 (detected by ELISA), serum pancreatic lipase, and amylase levels (measured using a fully automated biochemical analyzer).

5.4.2.2 Intestinal Function and Microbiota Parameters

Samples were collected at enrollment and on day 7 of intervention for analysis, including plasma DAO (measured by enzyme-coupled rate assay), plasma D-lactate levels (measured by spectrophotometry), and fecal p-cresol and indole concentrations (measured by gas chromatography-mass spectrometry [GC-MS]).

5.4.2.3 Clinical Outcome Measures

Including mechanical ventilation duration, ICU hospitalization duration, total hospitalization duration, incidence of infection complications (ventilator-associated pneumonia, catheter-related urinary tract infection, catheter-related bloodstream infection), degree of muscle atrophy on day 14 post-intervention (measured by ultrasound assessment of rectus femoris muscle thickness), and all-cause mortality at day 28 (the proportion of deaths occurring within 28 days after enrollment relative to the total enrolled population).

5.4.2.4 Feeding tolerance indicators

Record the incidence and duration of diarrhea (defined as ≥3 bowel movements per day with loose stool, excluding infectious diarrhea), abdominal distension (assessed based on abdominal physical examination and patient complaints), and gastric retention (retention volume>200 mL/4 hours) during the intervention period. Adverse reactions were classified into three grades according to severity: mild (no intervention required, duration <24 hours), moderate (requiring adjustment of infusion rate/dose or symptomatic management), and severe (requiring suspension of enteral nutrition support and initiation of specialized intervention).

5.4.2.5 Safety Indicator

Safety evaluation indicators primarily include the incidence of adverse reactions related to enteral nutrition (e.g., vomiting, aspiration, upper gastrointestinal bleeding), the incidence of allergic reactions (including pruritus, rash, dyspnea), and the incidence of abnormalities in liver and kidney function parameters (alanine aminotransferase [ALT], aspartate aminotransferase [AST], serum creatinine, blood urea nitrogen [BUN]) before and after intervention. All adverse reactions must be meticulously documented with details on occurrence time, severity, management measures, and clinical outcome.

**5.5 Follow-up Plan**

5.5.1 Baseline period (within 24 hours of enrollment)

The system systematically collects demographic data (age, gender, height, weight, BMI, etc.), clinical baseline information (types of underlying diseases, APACHE II score, SOFA score, surgical history, sepsis diagnosis, etc.), laboratory baseline parameters (pancreatic enzyme levels, liver and kidney function, complete blood count, baseline plasma amino acid levels), and gastrointestinal functional status prior to initiation of enteral nutrition (gastric retention volume, bowel sound intensity, etc.) from study subjects, establishing a comprehensive baseline database.

5.5.2 Intervention period (days 1, 3, 7, and 14 of the intervention)

Daily record the infusion rate, infusion dose, and feeding tolerance (including symptoms such as diarrhea, abdominal distension, or gastric retention); collect blood and stool samples on days 3, 7, and 14 of the intervention respectively and send them to the laboratory for endpoint parameter analysis (including amino acid metabolomics profiles, pancreatic function parameters, intestinal barrier function indicators, etc.); on day 14 of the intervention, assess muscle atrophy severity by measuring femoral straight muscle thickness via ultrasound; closely monitor patients' clinical conditions throughout the study period and promptly document any adjustments to therapeutic interventions.

5.5.3 Follow-up period (28 days after enrollment)

Trained research assistants conducted follow-up visits via telephone, meticulously documenting the survival status, residence location (family home/rehabilitation facility/hospital), and recurrence of infection in study subjects to ensure the authenticity and completeness of follow-up data. For patients who died during the follow-up period, the time and cause of death were recorded.

5.5. 4 Follow-up Responsibilities

Designate dedicated follow-up personnel and establish a follow-up record system to analyze and document the reasons for lost-to-follow-up cases, ensuring a follow-up success rate of ≥90%. During follow-up procedures, strictly protect patient privacy; all follow-up data shall be promptly entered into the research database and verified.

### 5.6 Sample Collection and Processing

5.6.1 Blood sample

Collect 5 mL of fasting venous blood from the subject, place it in an EDTA anticoagulant tube, gently invert and mix thoroughly, then centrifuge at 3000 rpm for 10 minutes at 4°C to separate the plasma, which is divided into two tubes (1 mL each) and rapidly stored in a-80°C ultra-low temperature freezer for analysis of amino acid metabolomics, DAO, D-lactate, and other parameters; simultaneously collect 3 mL of serum sample, place it in a standard dry test tube, allow it to stand at room temperature for 30 minutes, then centrifuge to isolate the serum for biochemical assays such as pancreatic enzyme activity and liver/kidney function tests.

5.6.2 stool specimen

Collect 5 g of fresh fecal samples from the study subjects, place them in a sterile cryovial, divide into two tubes (each containing 2 g), and immediately store them at −80°C in an ultra-low temperature freezer for detection of fecal elastase-1 and intestinal microbiota co-metabolites (p-cresol, indole).

5.6.3 Urine sample

Collect 24-hour urine samples from subjects, measure the volume, thoroughly mix the sample, and aspirate 10–20 mL of urine into a transfer tube using a sterile pipette. Immediately add 0.1 mL of concentrated hydrochloric acid (as a preservative), then rapidly store the sample in an ultra-low temperature freezer at −80°C for determination of urinary 3-methylhistidine concentration.

5.6.4 Sample Transportation

Each research center shall collectively transport frozen samples via cold chain logistics every month, maintaining the transportation temperature at-20°C. During transit, repeated freeze-thaw cycles must be strictly avoided, with detailed records kept of transportation temperature, duration, and sample quantity to ensure sample integrity.

5.6.5 Detection Process

Plasma amino acid concentrations, plasma citrulline concentration, and urinary 3-methylhistidine concentration were determined using LC-MS/MS; intestinal microbiota co-metabolites were analyzed by GC-MS; fecal elastase-1 was measured by ELISA; serum pancreatic lipase and amylase levels were measured using a fully automated biochemical analyzer; plasma DAO and D-lactate levels were determined respectively by enzyme-coupled rate assay and spectrophotometry.

## 6. Statistical Analysis Technique

This study employed the SPSS 26.0 and R 4.4.0 statistical software for all data analysis, with the entire process strictly adhering to the STROBE Declaration guidelines (for cohort studies). All statistical tests were conducted using two-sided comparisons, with $P<0.05$ considered statistically significant. All analyses were performed based on the intention-to-treat (ITT) principle, utilizing the full dataset of patients who met inclusion criteria and received at least one nutritional intervention. Additionally, per protocol completion (PP) analysis was performed for patients who completed the entire treatment course.

Sensitivity validation was performed using analyses of intervention and follow-up data from patients without serious protocol violations. By comparing the results of both analyses, the robustness and reliability of the study findings were ensured, thereby minimizing the impact of bias on the conclusions.

6.1 descriptive statistics

Quantitative data: First, normality (Shapiro-Wilk test) and homogeneity of variance were assessed. Data conforming to a normal distribution with homogeneous variances were expressed as mean ± standard deviation (X ± S); non-normal data were presented as median (interquartile range, IQR) [M (Q 1, Q3)]. Categorical data were expressed as frequencies (percentage, n/%).

6.2 comparison among groups

6.2.1 Measurement data: For comparisons between two groups, the independent samples t-test was used for data conforming to normal distribution with homogeneous variance, while the Wilcoxon rank-sum test was employed for skewed distributions. For repeated-measurement data (e.g., plasma amino acid concentrations or pancreatic enzyme levels at different time points), repeated-measurement ANOVA was applied for normally distributed data and the Friedman test for skewed distributions, with simultaneous analysis of time effects, group effects, and their interactions.

6.2.2 Count data: Comparison between the two groups was performed using the $\chi^2$ test; when the sample size is <5 or the theoretical frequency is <1, the Fisher exact test was employed.

6.3.2 Classification data: including adverse reaction severity and disease severity grading, etc., were compared between groups using the Wilcoxon rank-sum test.

6.3 Multivariate Analysis

6.3.1 Multiple linear regression model: Using protein absorption efficiency (plasma BCAA AUC −4 h) as the dependent variable and formulation type (short peptide group = 1, whole protein group = 0) as the primary independent variable, the model incorporated potential confounding factors—including age, APACHE II score, pancreatic enzyme levels, underlying disease types, and nutritional infusion doses—as covariates. After adjusting for these confounders, the impact of formulation type on protein absorption efficiency was evaluated.

6.3.2 Multivariate logistic regression model: Using the occurrence of infection complications and 28-day all-cause mortality as dependent variables, respectively, and protein absorption efficiency (categorized by quartiles) as the primary independent variable, the association between protein absorption efficiency and clinical outcomes was analyzed after adjusting for potential confounding factors, with odds ratios (OR) and 95% confidence intervals (CI) calculated.

6.3.3 Cox proportional hazards regression model: A single-factor regression analysis was first performed to identify variables with P <0.1 (e.g., age, APACHE II score, types of PEI high-risk factors, comorbid conditions, and concomitant medications). These variables were then incorporated into a multivariate regression model to adjust for potential confounding factors, analyze the associations between drug formulation type and protein absorption efficiency with 28-day all-cause mortality, calculate hazard ratios (HR) and 95% confidence intervals (CI), plot Kaplan-Meier survival curves, and compare survival curve differences between the two groups using the Log-rank test.

Assess the impact of different intervention measures on patients' short-term prognosis.

6.4 Subgroup Analysis and Sensitivity Analysis

6.4.1 Subgroup analysis: Stratified analysis was conducted based on high-risk causes of PEI (severe acute pancreatitis, postoperative abdominal conditions, sepsis) and pancreatic enzyme levels (normal, mildly elevated, moderately elevated) to investigate differences in protein absorption efficiency between the two EN formulations across subgroups and identify the appropriate patient populations for each formulation selection.

6.4.2 Sensitivity analysis: Perform statistical analysis again after excluding participants who were lost to follow-up to verify the stability of the study results; Conduct analyses using different confounding factor adjustment models (full model vs. stepwise regression model) to evaluate the impact of confounding factor adjustments on the results; Replace the primary outcome measure (e. g., use plasma citrulline concentration instead of BCAA AUC −4 h) and repeat the analysis to further validate the reliability of the study conclusions.

6.5 Data Missing Value Handling

For data with a missing rate <5%, the single-variable imputation method (e.g., mean or median imputation) is employed; for data with a missing rate of 5%–20%, multiple imputation is applied; for data with a missing rate>20%, a cause-of-missing analysis is conducted, and sensitivity analysis is performed to assess the impact of missing data on research outcomes.

## (II)Innovation and scientific value, key scientific problems and technical challenges to be addressed, risk assessment and control (including risk identification, risk control measures, risk-benefit evaluation, etc.)

### 1. Innovation and Scientific Value

This study focuses on comparing the differences in protein absorption efficiency between short-peptide-based and whole-protein-based enteral nutrition formulations in high-risk patients with postoperative ileus (PEI) on the intensive care unit (ICU). Addressing existing research gaps and clinical challenges, it identifies four core innovations that represent both academic breakthroughs and clinical translational value:

#### 1.1 Innovativeness

##### 1.1.1 Population Innovation

For the first time, this study precisely targeted individuals with a dual pathological condition of " acute stress in the ICU + high-risk for PEI." By integrating multidimensional indicators such as the APACHE II score, underlying comorbidities, and pancreatic enzyme levels, a standardized inclusion criteria system was established to define the core characteristics of the study population. This approach fills a research gap in nutritional intervention for high-risk acute PEI patients in the field of critical care medicine and distinguishes it markedly from previous studies focusing on general ICU patients or chronic PEI patients.

##### 1.1.2 Innovation of Method

Previous ICU nutrition studies primarily focused on "feeding tolerance and nutritional adequacy rates" as core indicators, with protein absorption assessment relying on macroscopic methods such as nitrogen balance and plasma total amino acids. These approaches failed to distinguish between the three fundamental mechanisms of "insufficient pancreatic enzyme digestion, intestinal absorption disorders, and hyperglycemic catabolism," nor could they adequately account for the pathological characteristics of high-risk populations with PEI. This study pioneered the integration of targeted amino acid metabolomics, gut microbiota co-metabolomics, and traditional evaluation metrics in high-risk ICU patients with PEI, establishing a comprehensive precision assessment system encompassing " pancreatic function − protein digestion − molecular absorption." Specifically: fecal elastase-1 and pancreatic lipase levels were quantified to assess exocrine pancreatic function; plasma essential amino acids (BCAA) and citrulline (a marker of intestinal epithelial absorption) precisely reflected absorption efficiency; urinary 3-methylhistidine (indicating muscle catabolism) and fecal p-cresol/indole (metabolites of non-absorbed proteins by gut microbiota) served as inverse validation markers for absorption defects. This approach achieves precise molecular-level quantification of protein absorption efficiency, overcoming the limitations of conventional macroscopic evaluation techniques and enabling more accurate identification of absorption differences between the two formulations.

#### 1.1.3 Design Innovation

This study employed a multicenter, prospective, randomized controlled trial design that not only reflected real-world clinical practice scenarios (randomized grouping without mandatory interventions, consistent with standard clinical protocols) but also ensured research rigor through stringent adjustment for confounding factors and single-blind assessment of outcomes. Additionally, dynamic follow-up was conducted over 7−14 days to enable continuous evaluation of absorption efficacy and clinical outcomes. The study provides robust evidence with strong clinical generalizability.

#### 1.1.4 Mechanism Innovation

This study, by correlating pancreatic function indicators, intestinal barrier function (plasma DAO and D-lactate), intestinal microbiota metabolic profiles, and protein absorption biomarkers, systematically elucidated for the first time the core pathological pathway: "insufficient pancreatic enzyme secretion → impaired whole protein digestion → fermentation of undigested proteins in the colon → reduced absorption efficiency." It further clarified the molecular mechanism by which short-peptide formulations circumvent pancreatic enzyme deficiency and enhance absorption efficiency via the intestinal PepT1 transporter, providing a theoretical foundation for formula optimization and personalized intervention of specialized nutritional preparations for severe cases.

### 1.2 Scientific Value

#### 1.2.1 Academic Value

This study enriches the theoretical framework for precision intervention in critical care nutrition, providing standardized detection methods and evaluation systems for assessing protein absorption efficiency in high-risk populations undergoing parenteral enteral nutrition (PEI) on the ICU. It advances the academic transition from "empirical nutrition" to "molecular precision nutrition" in the field of critical care nutrition and offers methodological references for subsequent research.

#### 1.2.2 Clinical Value

This study clarifies the applicable scenarios of two commonly used EN formulations—short peptide-based and whole protein-based—for high-risk patients undergoing ICU PEI, providing high-quality evidence-based medical data to support clinicians in developing personalized nutritional support plans. These findings contribute to reducing complications such as muscle atrophy and infections in patients.

Reduce the risk of disease occurrence and improve patient prognosis.

**1.2.3 Application Value**

The research findings provide essential data support for revising critical nutrition-related guidelines and expert consensus documents, guiding primary hospitals' ICUs in implementing standardized nutritional management for high-risk populations undergoing parenteral enteral nutrition (PEI), enhancing the overall standard of critical nutrition therapy in China, and reducing healthcare resource consumption.

## 2. Key Scientific Problems to Be Addressed

2.1 What are the core pathophysiological mechanisms underlying protein absorption disorders in high-risk populations with ICU PEI? How do the interplay among insufficient pancreatic enzyme secretion, impaired intestinal barrier function, and increased muscle catabolism regulate protein digestion and absorption efficiency, and what is the central regulatory node involved?

2.2 Are there statistically and clinically significant differences in protein absorption efficiency between short-peptide and full-protein EN formulations among ICU patients at high risk of PEI? Does this difference exhibit heterogeneity across subgroups with varying disease severity or different etiologies of PEI high risk?

2.3 Can improving protein absorption efficiency directly enhance clinical outcomes in high-risk populations with PEI in the ICU (e.g., reducing mechanical ventilation duration, lowering the incidence of infectious complications, and decreasing 28-day all-cause mortality)? What are the underlying mechanisms involved?

2.4 What is the gastrointestinal tolerance of short-peptide enteral nutrition formulations in high-risk populations for ICU PEI? Can they reduce the incidence of gastrointestinal adverse reactions, and is there a correlation between their tolerability mechanism and pancreatic function protection?

2.5 Does the short-peptide enteral nutrition formulation have a protective effect on pancreatic exocrine function in high-risk patients with postoperative pancreatitis (PEI) in the ICU, and is its impact on pancreatic function sustainable?

## 3. Technical Challenges and Solutions

**3.1** The precise quantification of protein absorption efficiency is challenging due to limitations of traditional evaluation metrics (e.g., nitrogen balance), which are susceptible to interference from errors in urinary and fecal nitrogen collection; standardization of metabolomic assays is difficult, and results across different testing batches exhibit poor comparability. Solutions include: employing LC-MS/MS for determining plasma amino acid concentrations, combined with cross-validation using indicators such as nitrogen balance and plasma citrulline concentration; establishing unified testing standards in core laboratories, providing centralized training and assessment for all analysts, and conducting regular internal quality control and external proficiency testing to ensure result reliability.

**3.2** In multicenter studies, control for confounding factors is essential due to variations in diagnostic and treatment protocols, preferences for EN formulations, and clinical practice habits across different research centers, which may lead to baseline confounding and compromise the validity of study outcomes. Solution: Establish standardized inclusion/exclusion criteria.

In addition to establishing standards and standardized clinical procedures (SOPs), unified training and assessment are conducted for researchers across all centers to ensure standardized implementation of studies. A multi-factor regression model is employed to account for potential confounding factors such as center effects, baseline disease severity, and underlying comorbidities, with subgroup analyses performed to verify consistency of study outcomes across different centers.

**3.3** The loss-to-follow-up rate during the follow-up period needs to be controlled. After discharge, ICU patients are often transferred to rehabilitation facilities or placed for home recovery, making contact information prone to change and increasing follow-up difficulty; an excessively high loss-to-follow-up rate may compromise the reliability of research results. Solutions: Establish a dedicated follow-up system; collect multiple contact details (phone numbers, WeChat IDs, detailed residential addresses, etc.) from participants and their families at enrollment, and obtain signed informed consent forms for follow-ups; remind participants by phone or WeChat one day prior to follow-up; combine telephone and home visits for follow-up; analyze reasons for patient loss-to-follow-up, and expand the sample size when necessary to mitigate its impact.

## 4. Risk Assessment and Management

4.1 Sample Size Risk Management: Each research center shall designate a dedicated enrollment officer responsible for subject screening and enrollment, reporting monthly progress; the project team shall hold regular multicenter investigator meetings to promptly analyze reasons for slow enrollment rates and adjust strategies accordingly; During enrollment, subjects must be strictly screened according to inclusion/exclusion criteria, avoiding mandatory enrollment of non-compliant patients to accelerate enrollment speed; When estimating sample size, reserve 20% as buffer to account for potential loss to follow-up; Establish an enrollment incentive mechanism to enhance researchers' motivation across centers.

4.2 Ethical Risk Management: All research centers must obtain approval from their respective Institutional Review Boards (IRBs) prior to initiating any study and retain the corresponding approval number; any revisions to the study protocol require re-approval by the IRB; For subjects with impaired consciousness, a signed informed consent form must be obtained from their legally authorized family members, with detailed explanations provided regarding the study objectives, procedures, potential risks, and benefits prior to signing to ensure full understanding and voluntary consent; An adverse event response plan shall be established, specifying clear criteria for identifying severe adverse events and corresponding management protocols; in the event of a severe adverse event, intervention shall be immediately terminated, standard clinical care administered, and the incident promptly reported to both the IRB and the project team.

4.3 Detection Risk Management: Establish unified standardized operating procedures (SOPs) for sample collection, storage, and transportation; provide specialized training and assessment to researchers at all centers, with sample processing permitted only after passing the assessment; Ensure core laboratories are uniformly equipped with testing instruments and reagents, perform regular equipment calibration and reagent quality control, conduct internal quality control (by testing daily reference standards, allowing sample analysis only when results fall within permissible ranges), and participate in external quality assessments organized by the National Health Commission to ensure the accuracy and comparability of test results.

Keep a portion of backup samples for repeat testing and verification if abnormal results are detected.

4.4 Bias Risk Management: The propensity score matching (PSM) method was employed to correct baseline imbalance between the two study groups, ensuring comparability of baseline characteristics; A single-blind design for outcome assessment was strictly implemented, with samples and data managed through coding, and neither investigators nor statistical analysts were aware of group assignments; Data entry followed a dual-entry system, followed by logical verification and error correction to ensure accuracy.

4.5 Clinical Risk Management: EN infusion should strictly adhere to the principle of gradual progression; the initial infusion rate should not be too rapid, and the patient's feeding tolerance must be closely monitored with timely adjustments to the infusion regimen; In cases of adverse reactions such as diarrhea or vomiting, initial measures include slowing the infusion rate and administering symptomatic treatment; if ineffective, intervention may be discontinued and management should follow withdrawal criteria; Daily assessment by the attending physician is required.

Monitor the patient's condition changes promptly, documenting disease progression and research intervention measures. In cases of sudden deterioration, prioritize emergency treatment before recording relevant study information as required.

### 5. Risk-Benefit Assessment

This study was a randomized controlled trial that employed clinically commonly used short-peptide and whole-protein EN formulations as intervention measures, without any additional invasive procedures. The study protocol strictly adhered to clinical practice guidelines. The primary research risks were mild adverse reactions associated with enteral nutrition (e.g., diarrhea, abdominal distension), which occurred at low incidence and could be effectively managed with symptomatic treatment; overall, the study risks remained manageable.

The study yielded significant and multidimensional benefits: For patients, it provides more targeted nutritional support regimens that effectively improve protein absorption, reduce the risk of complications such as muscle wasting and infections, thereby enhancing prognosis; Clinically, it fills the evidence gap in nutritional intervention for high-risk populations undergoing parenteral enteral nutrition (PEI) in the ICU, offering physicians a scientific basis for precise selection of enteral nutrition (EN) formulations and optimizing critical care nutrition management protocols; Academically, it advances technological innovation and theoretical development in critical care nutrition, providing methodological references and data support for future research. Overall, the clinical and academic benefits of this study far outweigh its potential risks.

## (III) Data Management and Quality Control Plan

### 1. Data Management

1.1 Data Collection: Data were collected using a uniformly designed electronic case report form (eCRF), which included demographic information, baseline clinical data, intervention details, laboratory test results, and follow-up outcome data of study subjects. Data entry was performed by trained researchers at each center; all entries must be timely, accurate, and complete, with missing values clearly labeled to ensure traceability.

1.2 Data Verification: Dual-entry verification: Each eCRF is independently entered by two researchers; after entry, data is compared, and any discrepancies are promptly verified against the original records for correction; Logical verification: The eCRF system incorporates unified logical rules (e.g., age ranges, APACHE II score intervals, normal reference ranges for test parameters), with automatic alerts triggered for abnormal data, which are then confirmed by the data administrator; Regular verification: The data administrator conducts quarterly random checks on data entered by each center (with a minimum coverage rate of 10%); identified issues are immediately reported to the respective centers for rectification.

1.3 Data Storage and Confidentiality: Electronic data are stored on encrypted servers, with the server password jointly managed by the data administrator and the project lead, and regular backups are performed (at least once per week). Paper-based materials (including informed consent forms, original laboratory test reports, research records, etc.) are custodied by designated personnel at each center and stored in locked filing cabinets for a retention period of 5 years after study completion. All research data shall be used exclusively for this study and strictly comply with the Helsinki Declaration and China's medical data regulations.

In accordance with confidentiality regulations, the privacy of research subjects must be protected, and data sharing requires joint approval from both the project leader and the ethics committees of each participating center.

1.4 Data Locking and Unlocking: Upon completion of the study, all research data shall be jointly reviewed by the data administrator, statistical analyst, and project lead. Once data completeness and accuracy are confirmed, the data shall be locked; no modifications may be made to locked data. In exceptional circumstances (e.g., identification of significant data entry errors), unlocking of data requires submission of a written request detailing the justification. Following approval by the ethics committee, the data administrator and project lead shall jointly unlock and modify the data, with detailed documentation of the modification rationale, content, and date.

## 2. Quality Control Plan

### 2.1 Personnel Training

Prior to initiating the study, all researchers at each center (including physicians, nurses, laboratory technicians, and data entry personnel) underwent standardized training covering the interpretation of the study protocol, definition of inclusion/exclusion criteria, eCRF entry protocols, sample collection and processing procedures, follow-up protocols, fundamentals of statistical methods, and ethical compliance requirements. Following the training, participants underwent both theoretical and practical assessments; only those who passed were eligible to participate in the study. Regular retraining was conducted throughout the study to ensure adherence to standardized operational practices by researchers.

### 2.2 Central Quality Control

Each research center appoints dedicated quality control officers responsible for overseeing research quality assurance within their respective centers. These officers conduct daily checks on the eligibility of enrolled cases, the accuracy and completeness of eCRF entries, and the compliance of sample collection and storage protocols. They prepare monthly quality control reports and submit them to the project team to promptly identify and address any issues arising during the implementation of research activities at their centers.

### 2.3 Comprehensive Supervision Throughout the Process

The project team established a Supervision Committee composed of experts in critical care nutrition and statistics. This committee conducts on-site inspections at each research center every six months to evaluate study progress, data quality, sample storage and transportation conditions, as well as the implementation of quality control measures. For issues identified during supervision, the committee provides corrective recommendations and monitors their implementation status.

### 2.4 Laboratory Quality Control

The core laboratory has established standardized testing procedures, regularly calibrates and maintains testing equipment, conducts quality inspections of reagents to ensure that both equipment and reagents meet testing requirements; performs daily internal quality control by testing control samples and recording results. If control results exceed permissible limits, sample testing is immediately halted, issues are investigated and resolved, and testing resumes; participates periodically in external quality assessments organized by the National Health Commission to ensure the accuracy and comparability of test results.

**2.5 Ethical Quality Control**

The project team regularly reports research progress and adverse reaction occurrences to the ethics committees of each center. If the study protocol requires revision, it must be resubmitted for ethical approval and can only be implemented upon approval. The team strictly adheres to ethical review recommendations to safeguard the legitimate rights, interests, and safety of study participants.

**2.5 Compliance Nature Control**

Strictly adhere to Good Clinical Practice (GCP) and the relevant research management regulations of each center. Maintain comprehensive documentation throughout the entire study process; all research records, test reports, quality control reports, and other materials must be properly preserved to ensure compliant execution of the study and guarantee the reliability of the results.

## (IV) Principal Investigators and Team Responsibilities

### 1. Principal Investigator

Professor Wang Sheng, Department of Critical Care Medicine, Tongji Hospital Affiliated to Tongji University

Professor Qu Hongping, Department of Critical Care Medicine, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

Professor Fang Zongping, Department of Critical Care Medicine, The Fourth People's Hospital Affiliated to Tongji University

### 2. Division of Labor of the Team

This project is based at Tongji Hospital affiliated with Tongji University, with collaborative partners including Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine and the Fourth People's Hospital affiliated to Tongji University. A Clinical Research Collaboration Group Expert Committee composed of principal leaders from all member institutions has been established to formulate annual work objectives, oversee the smooth progress of tasks through regular meetings, and promptly address issues and challenges identified by the collaborating units.

Main responsibilities of the lead unit: Establish and improve project-related management systems and oversee their implementation; In accordance with the specific project implementation plan, proactively collaborate with all participating units to steadily advance all tasks; Enhance training and technical guidance for project participants; Regularly monitor and provide feedback on the progress of work across all collaborating units; Develop an information system for the collection, analysis, monitoring, and management of clinical data related to the project; Hold regular research progress meetings to discuss project execution progress, quality issues, existing challenges, and solutions; Provide necessary human, material, and financial support for project implementation and management.

Main responsibilities of the collaborating unit: Cooperate with the lead unit in establishing and improving project-related management systems and implement them accordingly; diligently advance all tasks in accordance with the specific project implementation plan; regularly monitor the progress and quality of completion of project tasks within the unit; periodically report project progress to the lead unit; ensure proper submission of relevant information and data and secure original documentation; support project implementation and management.

Provide the necessary support conditions in terms of human resources, material resources, financial resources, etc.

The specific responsibilities of each member unit are as follows: Head of the Collaboration Group: Responsible for proposing major research topics and coordinating affairs among collaborating units; Experts of the Collaboration Group: Appointed by the heads of each collaborating unit, responsible for overseeing the implementation, monitoring, and providing feedback on specific research activities; Clinical Researchers: Chief clinical physicians from each member unit, responsible for conducting research, collecting data, reporting events, and providing feedback and recommendations; Health Statistics Experts: Responsible for assisting in developing research protocols, guiding data analysis, and reviewing data quality.

## III. Annual Progress of the Project and Annual Performance Evaluation Criteria

| Year | The project's annual plan and objectives (with work milestones defined by quarter, specifying key, mandatory milestone targets) |
|---|---|
| Fiscal Year 2026 | First quarter: Refine the research protocol and convene an expert advisory meeting to complete the revision of the protocol justification; all participating centers shall submit ethical review applications simultaneously.<br>Second quarter: Develop a unified research protocol, data collection standards, and quality control plan; conduct centralized training and assessment for all researchers.<br>Third quarter: Completed ethical approval and clinical study registration; Concluded kick-off meetings for clinical studies at all participating institutions; Established an EDC data collection system and completed debugging and trial operation.<br>Fourth quarter: Screen eligible study participants according to inclusion/ exclusion criteria and complete enrollment agreements; systematically collect baseline data (demographic characteristics, underlying diseases, etc.) from participants and promptly enter them into the EDC system. |
| Fiscal Year 2027 | First quarter: All research centers continued to screen study subjects, systematically collected baseline data (demographic characteristics, underlying diseases, etc.) and promptly entered it into the EDC system.<br>Second quarter: Each research center continued to screen study subjects, systematically collected baseline data (demographic characteristics, underlying diseases, etc.), and promptly entered the data into the EDC system.<br>Third quarter: All research centers continued to screen study subjects, systematically collected baseline data (demographic characteristics, underlying diseases, etc.) and promptly entered it into the EDC system; completed the cleaning, verification, and locking of all research data, and prepared a data cleansing report.<br>Fourth Quarter: The statistical team completed various statistical analyses using designated software according to predefined protocols, authored academic papers, and submitted them for publication; compiled data from the entire research process, summarized research findings, and promoted these outcomes through academic conferences and specialized training sessions to facilitate clinical translation. |