Effects of Late-evening Snacks on Nutritional Status and Metabolic Pattern of Patients With Primary Hepatocellular Carcinoma After Hepatectomy

Source：Drum Tower Hospital, Medical School of Nanjing University

Subject No.: None

Person in charge: Xiaojie Bian, Yao Du

Department: Pharmacy

Contact: Xiaojie Bian, Yao Du

TEL: 86-18061678828, 86-18351887978

Study start date: February 2024

Version number: V2.0

Version date: December 22, 2023

Produced by Drum Tower Hospital, Medical School of Nanjing University

Formula Case Abstract Summary

Project name	Effects of Late-evening Snacks on Nutritional Status and Metabolic Pattern of Patients with Primary Hepatocellular Carcinoma After Hepatectomy
Research purpose	To observe the effects of Late-evening Snacks on the nutritional status, liver function recovery, complication rate and long-term prognosis of hepatocellular carcinoma patients who underwent hepatectomy, and to further explore the effects of Late-evening Snacks on the metabolic pattern of the patients, and to identify the serum differential metabolites, in order to initially investigate the potential mechanism of Late-evening Snacks to improve the nutritional status of the patients, and to provide theoretical basis for the development of an effective nutritional regimen in the clinic.
Research design	Single-center prospective cohort study
Total Number of Cases	106 cases
Case selection	***Inclusion Criteria:*** 1. Age 18-75 years; 2. Meet the diagnostic criteria of China's "Guidelines for Diagnosis and Treatment of Primary Liver Cancer (2022 Edition)", clinically diagnosed with primary liver cancer, hospitalized with radical hepatectomy as the main surgical treatment, no indication of metastasis of the tumor to extra-hepatic organs in preoperative tests and examinations, no absolute contraindications to surgery, complete resection of the liver tumor in the operation, and hepatocellular carcinoma confirmed by postoperative pathological diagnosis； 3. Child-Pugh grades A and B; 4. Preoperative Eastern Cooperative Oncology Group Physical Status Score

	(ECOG-PS) of 0 to 2; 5. The patient is conscious, has normal verbal communication, and is able to cooperate with the relevant examinations; 6. Fully informed about the study and voluntarily signed an informed consent form.
	Exclusion Criteria: 1. Failure to meet selection criteria; 2. Nutritional assessment as cachexia; 3. Presence of contraindications to enteral nutrition (EN) or EN intolerance, such as acute gastrointestinal bleeding, intestinal obstruction.(≥ grade 3, National Cancer Institute-Common Terminology Criteria for Adverse Events [NCINCI-CTCAE v 5.0]); 4. Simultaneous combination of malignant tumors in other parts of the body; 5. Combined hepatic encephalopathy or definite infection on admission; 6. Known refractory metabolic diseases (e.g., poorly controlled diabetes mellitus or fasting glucose ≥10 mmol/L, hyperthyroidism, hypothyroidism, metabolic acidosis); 7. Decreased renal function (defined as serum creatinine Cr level ≥176.8 μmol/L); 8. Intravenous or oral nutritional supplements, such as proteins, amino acids, etc., applied within one month prior to admission to the hospital; 9. Patients with severe stress or severe complications such as respiratory failure with severe cardiac, hepatic, renal and other insufficiencies； 10. Persons with mental and neurological disorders who are unable to cooperate with a physician; 11. Alzheimer's disease, cerebral atrophy, acute stage or sequelae of cerebrovascular disease, cognitive impairment; 12. Previously poor adherence to medication and nutritional counseling; 13. Critically ill and difficult to assess;

	14. On the liver transplant waiting list or under consideration for liver transplantation, as such patients may discontinue follow-up before the end of the study; 15. Less than 12 months since last localized treatment (TACE or HAIC or ablative therapy); 16. Other circumstances that the researcher considers inappropriate for participation in the study.
Treatment plan	Patients who were admitted to the Department of Hepatobiliary Pancreatic Surgery of Drum Tower Hospital affiliated to Nanjing University School of Medicine from February 2024 to January 2025 were selected and screened for enrollment according to the admission criteria. The grouping was not randomized and blinded, and all patients were enrolled according to their individual wishes and divided into a test group (53 cases) and a control group (53 cases). The purpose of the study, the content of the investigation were explained to the patients or their families at the time of admission, the principle of confidentiality was informed to reduce the concerns of the respondents, and the subjects were included in the study after signing an informed consent form after obtaining their consent. 1. Treatment programs All patients were screened for nutritional risk within 24h of admission using NRS 2002, nutritional assessment using PG-SGA, and malnutrition diagnosis using GLIM malnutrition assessment (diagnosis) criteria. A target daily amount of 25-30 kcal/kg was set for the patients. Nutritional management was standardized to ensure a balanced dietary intake in both groups. Nutritional cards were issued to each patient according to his/her intake, and were explained and emphasized at the time of admission and at the time of discharge. Patients were asked to choose different food items on the cards according to their own food preferences and actual situation. The experimental group was required to have late-evening snacks (LES) within

	1hour before bedtime, while the control group did not have any food during the 1hour before bedtime. **Test group:** patients will have an additional meal (total calories 200-275kcal, protein 11.5g-18g, complex carbohydrates 25-55g) 1h before bedtime. The dietary regimen of the test group will be subtracted from the corresponding late-evening snacks intake. (1) Preoperative: ①Patients without nutritional risk: independent intake according to the issued diet program card. ②Patients with nutritional risk: daily oral whole-protein enteral nutrition on the basis of general diet after admission. (2) Postoperative: start drinking small amount of water on the 1st postoperative day, after ventilation, over to liquid diet, and on the 2nd postoperative day, it can be over to autonomous semi-fluid diet + daytime oral enteral nutrition of whole protein type + LES regimen. As the patient's voluntary intake meets 60% of the body's needs, the daytime oral enteral nutrition support can be gradually stopped and the LES regimen can be continued. 3) Discharge: daily intake is required on time according to the LES regimen. **Control group:** no more food 1h before bedtime. (1) Preoperative: same as the test group. (2) Postoperative: start drinking small amount of water on the 1st postoperative day, after ventilation, over to liquid diet, and on the 2nd postoperative day, it can be over to autonomous semi-fluid diet + daytime oral enteral nutrition of whole protein type. As the patient's voluntary intake meets 60% of the body's needs, the daytime oral enteral nutrition support can be gradually stopped and the LES regimen can be continued. (3) Discharge: the doctor will judge whether the patient needs to continue nutritional support according to his/her dietary recovery, and if so,

	his/her supplementation must be completed before 1h before bedtime. 2. Biological sample collection: The remaining post-test serum samples need to be collected for metabolomics analysis from patients on day 1 of admission, postoperative day 6, postoperative 1 month, postoperative 3 months, and postoperative 6 months. 3. Clinical data collection: 1) General information: gender, age, height, weight, BMI, ICG 15min retention time, name of surgery, duration of surgery, blood loss, blood transfusion, tumor stage, NRS2002, GLIM malnutrition assessment (diagnostic) criteria diagnosis. 2) Nutritional indicators: Nutritional indicators such as serum albumin (ALB), prealbumin (PA), transferrin (TRF), and total serum protein (TP) were collected from patients on the 1st day of admission, the 6th postoperative day, 1 month postoperatively, 3 months postoperatively, and 6 months postoperatively. (3) Liver function indexes: Liver function indexes such as alanine aminotransferase(ALT), alanine transaminase(AST), γ-glutamyltransferase (GGT), cholinesterase (CHE), and total bilirubin (TBIL) were collected on the 1st day of admission, the 6th postoperative day, 1 month postoperatively, 3 months postoperatively, and 6 months postoperatively. (4) Clinical outcome: collect the patients' postoperative clinical outcome indicators, such as: postoperative complication rate, recurrence-free survival, and quality of survival. (5) Medical costs: collect the total cost of the patients' hospitalization, the cost of anti-infective drugs, and the cost of albumin during hospitalization.
Evaluation of the	*Indicators of effectiveness evaluation* Main efficacy indicators: serum albumin (ALB) Secondary efficacy indicators: serum prealbumin (PA), alanine

efficacy of treatment	aminotransferase (ALT), azelaic transaminase (AST), cholinesterase (CHE), total bilirubin (TBIL), total hospitalization costs, relapse-free survival, and quality of life
	Safety evaluation indicators Incidence of gastrointestinal symptoms such as abdominal distension, diarrhea, nausea and vomiting, incidence of postoperative complications, and length of postoperative hospitalization
Statistical methods	Descriptive statistics were performed on the clinical data, and measures that conformed to normal distribution were expressed as mean±standard deviation ($\bar{x}\pm s$), and t-test was used between groups, and measures that did not conform to normal distribution were expressed as median (interquartile spacing) [M(IQR)], and Mann-Whitney U test was used between groups. Count data were expressed as percentages [n(%)], and the chi-square test was used for between-group comparisons, with $P<0.05$ considered statistically significant. The Kaplan-Meier method in STATA 12.0 was used to calculate the recurrence rate and recurrence-free survival in each group, and the log-rank test was used for intergroup comparison.
Duration of the study	24 months

catalogs

1. Research background 1
2. Research purpose 4
3. Types of Research Designs and Experimental Steps 4
4. Case selection 9
5. Research Methodology and Technical Approach 10
6. Observed items and testing time points 12
7. Criteria for evaluating the efficacy of treatment 13
8. Adverse Events Watch 14
9. Data security monitoring 16
10. Statistical processing 16
11. Ethical principles of clinical research 17
12. Reference 17

1. Research background

Primary liver cancer (hereinafter referred to as hepatocellular carcinoma) is one of the common malignant tumors of the digestive system worldwide, which can be classified into hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and combined hepatocellular-cholangiocarcinoma (cHCC-CCA) according to the different pathologic types. According to the Global Tumor Epidemiology Statistics 2020 (GLOBOCAN), the number of newly diagnosed cases of hepatocellular carcinoma amounted to 906,000, which is the 6th most common type of cancer, and the number of deaths amounted to 830,000, which is the 3rd most common type of cancer [1]. And the incidence rate of liver cancer in China is located in the 4th place, the mortality rate is located in the 2nd place [2], and the 5-year survival rate is 14.1% [3]. The treatments for hepatocellular carcinoma vary according to the stage and liver function level, including hepatic resection, liver transplantation, ablation therapy, transarterial embolization, radiation therapy, etc. Among them, hepatic resection is considered to be the most effective treatment for early-stage hepatocellular carcinoma and is the main treatment at present, with a 5-year survival rate of up to 69.0%~86.2% [4]. Research results show that the local recurrence rate of selecting surgical resection treatment modality is significantly lower than that of radiofrequency ablation for hepatocellular carcinoma patients with good liver function reserve, and the long-term efficacy is better [5,6].

At the same time, the liver serves as the target organ for the metabolism of most nutrients, and the high metabolic status and organ invasion of cancer often expose liver cancer patients to malnutrition, with an incidence rate as high as 74.36% according to a survey [7]. In addition, hepatic resection will cause hepatocyte damage to a certain extent, resulting in the metabolic balance of sugar, protein, fat and hormones in the patient's body being disrupted, which makes the body's catabolism stronger than anabolism [8], and it is more likely to have a decrease in the level of albumin synthesis and a significant decrease in the intake of nutrients after the operation, which in turn results in the decrease in the patient's ability to resist infection, the increase in the risk of complications, and the decrease in the quality of life and a series of other poor prognosis [9,10,11,12], therefore, nutritional support and intervention are particularly important in this situation. According to our previous study, we found that nutritional intervention by clinical pharmacists in perioperative liver cancer hepatectomy patients could reduce the complication rate by 69%, as well as correct the inflammatory stress response and improve albumin levels more quickly [13].

Therefore, we believe that the use of rational and effective nutritional intervention strategies will bring positive benefits to patients.

The 2022 Guidelines for Perioperative Care in Liver Surgery: the Accelerated Rehabilitation After Surgery Society (ERAS) recommends early intake after hepatic resection and oral nutritional supplements for malnourished patients [14]. However, for patients with abnormal liver function or cirrhosis their perioperative and postoperative nutritional support and nutritional support patterns lack guideline recommendations. Abnormal liver function and inadequate intake in postoperative patients cause patients to exhibit reduced hepatic glycogen reserves, enhanced gluconeogenesis and fat oxidation, and increased protein oxidative catabolism. To mitigate accelerated starvation and associated protein hydrolysis, the most critical dietary strategy is to eat meals every 4 to 6 hours with the shortest possible interval between meals. Therefore, the concept of Late evening snack (LES), in which a portion of food is moved to bedtime while the total daily intake remains unchanged, has been proposed to effectively improve the metabolic state of accelerated catabolism and has been recommended by the European Society of Clinical Nutrition and Metabolism (ESPEN) for patients with cirrhosis who have a high catabolic metabolism[15] , without indicating the specific nutritional composition, while the International Society for Hepatic Encephalopathy and Nitrogen Metabolism recommended in its consensus published in 2013[16] that LES should contain at least 50 g of complex carbohydrates. In most studies, various combinations of LES (200-275 kcal total calories, 11.5g-18g protein, and 25-55g complex carbohydrates) have been shown to be significant in improving liver function indices and nutritional indices [17]. Researchers have suggested that LES containing complex carbohydrates and proteins may reduce lipid oxidation and improve nutritional status, muscle mass, liver function and quality of life in patients [18,19].

The results of a study in an outpatient long-term late-evening snacks also confirmed a significant improvement in nitrogen balance compared to baseline and an increase in serum albumin levels [20]. In a meta-analysis published in 2018 [19], serum albumin, prealbumin, and cholinesterase levels were significantly elevated in patients with cirrhosis after implementation of LES. In addition, Chen et al [17] found that LES reduced the levels of alanine aminotransferase (ALT), alanine aminotransferase (AST), prothrombin time (PT), and blood ammonia in patients with liver disease, and reduced the incidence of ascites. With further research, Takeshita et al [21] found that LES prevented the decline of albumin levels in hepatocellular carcinoma patients undergoing chemoembolization therapy (TACE) and reduced hepatic impairment due to TACE. Meanwhile, Harima et al [22] showed that LES improved prealbumin levels,

BCAA/tyrosine, and ALT in patients with advanced hepatocellular carcinoma treated with hepatic artery perfusion chemotherapy. In addition, Morihara et al [23] found that LES also significantly improved Child-Pugh score in hepatocellular carcinoma patients at 4 and 12 weeks after radiofrequency ablation.

The improvement of nutritional status of LES for liver cancer patients is currently limited to the detection of several biochemical biomarkers and anthropometric measurements, which lacks a comprehensive understanding of metabolic changes. At the metabolic level, metabolomics is an important component of systems biology that allows the analysis of metabolic changes in the organism as a result of relevant factors. Pathologic changes in the liver affect the body's basal metabolism and have a more pronounced effect on the levels and types of small molecule metabolites, which provides a new perspective for applying metabolomic analysis to study the changes in metabolic patterns in patients with LES. In recent years, metabolomics has also been increasingly used in dietary intervention studies to discover new dietary biomarkers and to explore the metabolic changes associated with nutrients and dietary patterns, in order to evaluate the effects of dietary interventions on the disease of the organism. Tripodi et al [24] found that methionine supplementation in vitro could inhibit AMP-activated protein kinase and up-regulate the tricarboxylic acid cycle, through proteomics and metabolomics to inhibit hepatocellular carcinoma invasion. Rong et al [25] investigated the effect of antioxidant active substance corn oligopeptides on serum metabolism of hepatocellular carcinoma patients based on metabolomics combined with multivariate statistics. Initially, 16 differential metabolites were screened, and it was concluded that corn oligopeptides' protection of hepatic function was realized through nicotinic acid and nicotinamide metabolism and fatty acid metabolism pathways. However, it is not clear how LES affects the metabolic patterns of patients to improve nutritional status and liver function. As a result, metabolomics has the potential to make an important contribution to the study of the underlying mechanisms of LES to improve the nutritional status of patients and to the search for methods of nutritional support in malignant tumors.

Since, nutritional support with LES for hepatocellular carcinoma patients undergoing hepatectomy treatment has rarely been reported, and there are no clinical studies applying metabolomics approaches to explore the effects of LES on their metabolic patterns. In view of this background, the present study aimed to optimize the management of meal timing and frequency in patients with hepatocellular carcinoma undergoing hepatectomy using LES, with the aim of reducing patient complications and improving clinical outcomes, and to further study and explore the effects of LES on patients' metabolic patterns to

provide a theoretical basis for implementing effective nutritional strategies in clinical practice to promote patient recovery.

2. Research purpose

2.1 Primary purpose

To observe the effects of late-evening snacks on the nutritional status, liver function recovery, complication rate and long-term prognosis of hepatocellular carcinoma patients who underwent hepatectomy, and to further explore the effects of late-evening snacks on the metabolic pattern of the patients through metabolomics, and to identify the serum differential metabolites, in order to initially investigate the potential mechanism of late-evening snacks to improve the nutritional status of the patients, and to provide theoretical basis for the development of an effective nutritional regimen in the clinic.

2.2 Secondary purpose

The sample size can be enlarged to continue the population study, to verify and continue to optimize the test results obtained from the validation, to shift from a single-center study to a multi-center study, and to establish an expandable multi-dimensional database. In order to propose a more reasonable nutritional support strategy for the clinic and provide reference for doctors' clinical decision-making.

2.3 Test hypothesis

It was hypothesized that there would be significant differences in serum albumin, prealbumin, and serum metabolic patterns in subjects in the test group compared to control patients, and potential mechanisms by which the LES would improve the nutritional status of patients would be identified.

3. Types of Research Designs and Experimental Steps

3.1 Types of Research Designs

Types of study designs: single-center, prospective cohort studies

Research Center: Drum Tower Hospital, Medical School of Nanjing University

Grouping method: non-randomized, open, enrolled according to patients' wishes

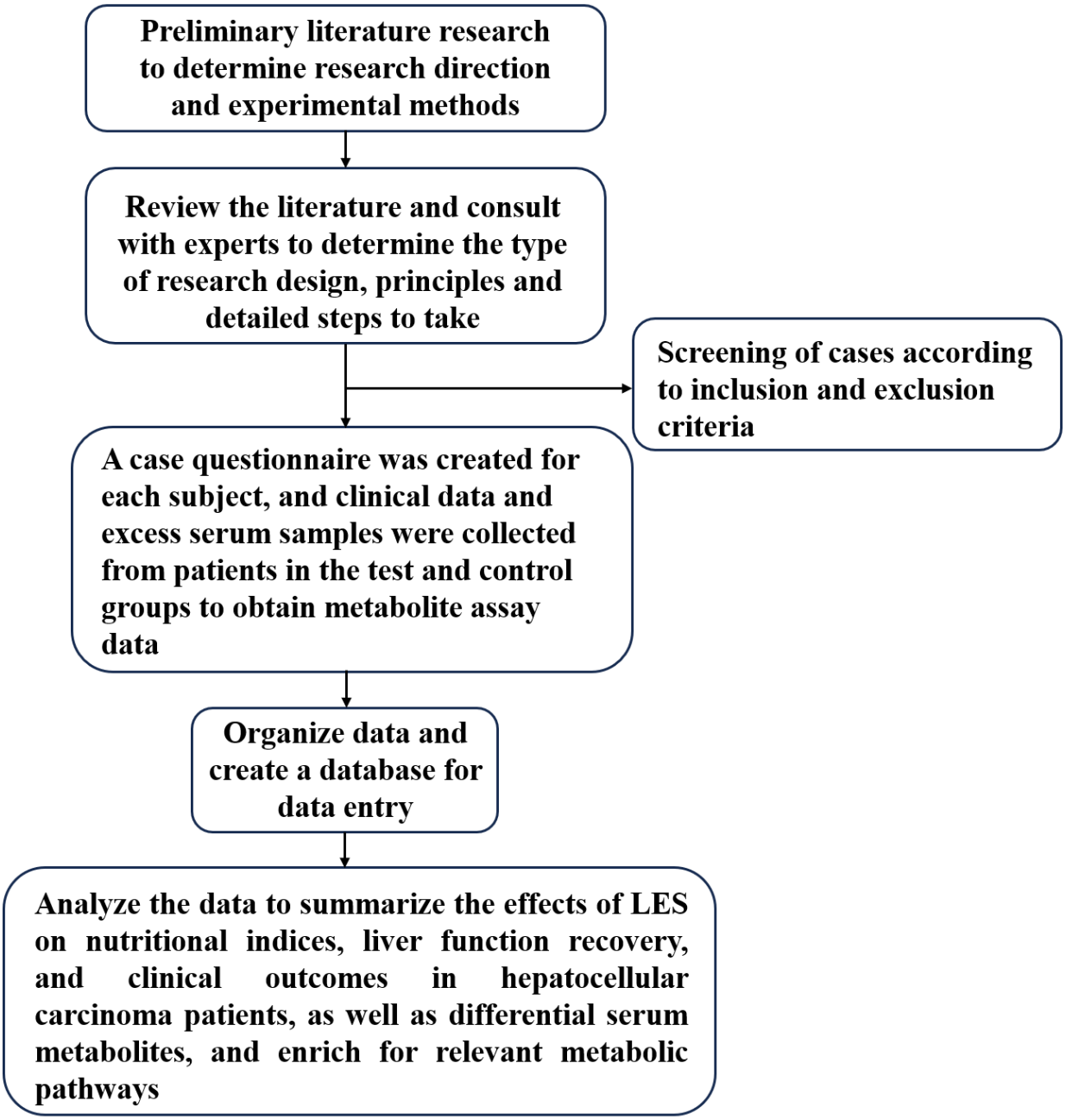
Sample size calculation basis and formula:

This study was a single-center prospective cohort study with two groups, the test group and the control group, and the serum albumin level of the study subjects was the primary endpoint outcome indicator observed. Based on the review of previous literature [28] and reference to the results of the pretest, it was expected that the albumin level before LES intervention was 34.2±3.9 g/L and the albumin level

after LES intervention was 37.5±5.4 g/L, with a two-sided α=0.05 and a degree of certainty of 1-β=90%. Using PASS15 software can be calculated to obtain n=88 cases. Based on the 1:1 grouping, 44 subjects were needed in each of the test and control groups, and assuming a 20% subject dropout rate, the final minimum number of subjects needed was 53 in each of the test and control groups, for a total of at least 106 subjects included.

3.2 Experimental Steps

The specific steps of the study are as follows:



Pre-procedure preparation:

According to the literature research, establish the form of patients' clinicopathological data that need to be collected, including basic information (age, gender, hospitalization number), pre-surgical laboratory examination (nutritional indexes, liver function, renal function, coagulation function, blood routine, tumor markers such as alpha-fetoprotein, etc.), surgical records (tumor location, size, number, intra-operative bleeding, etc.), post-surgical laboratory examination, tumor recurrence and treatment modality and patient survival, etc., as well as the establishment of a dietary matching table, respectively writing the dietary education content of the experimental group and the control group, in order to personalize the guidance of the patient's postoperative diet and the correct understanding of the importance of dietary therapy. A case questionnaire was set up for the enrolled patients, and the relevant data of the enrolled patients were recorded in the form.

Screening and Trial:

Patients who were admitted to the Department of Hepatobiliary Pancreatic Surgery of Drum Tower Hospital affiliated to Nanjing University School of Medicine, from February 2024 to January 2025 and met the enrollment criteria were selected for inclusion in this study. The grouping was not randomized and blinded, and all patients were enrolled according to their individual wishes and divided into a test group (53 cases) and a control group (53 cases). The purpose of the study, the content of the investigation, the principle of confidentiality were explained to the patients or their families at the time of their admission to the hospital to reduce the concerns of the respondents, and the subjects were included in the study after signing the informed consent form with their consent.

1. Treatment program:

All patients were screened for nutritional risk using NRS 2002, nutritional assessment using PG-SGA, and malnutrition diagnosis using GLIM Malnutrition Assessment (Diagnosis) Criteria within 24h of admission. In accordance with the recommendations of ESPEN 2021 Practice Guidelines for Surgical Nutrition Therapy and CSPEN 2016 Guidelines for Perioperative Nutritional Support for Adults, the patients' daily target amount was set at 25-30 kcal/kg.In order to ensure the balanced dietary nutritional intake of the two groups, their standardized management was formulated as follows:① Total calories: according to the patients' height, weight, age and activity Total calories: Calculate the daily intake according to the patient's height, weight, age and activity intensity, about 1200~2000 kcal. ② Carbohydrates: account for 60%~65% of the actual energy supply, calculate the actual supply of 180~325

g. The staple food is mainly cereals and potatoes. Protein: 1.2~1.5 g/kg/day according to the guideline, choose high quality protein, such as eggs, poultry, soybean products. ④Fat: account for 20% of the actual energy supply, calculate the actual supply of about 27~44 g. Distribute the energy of three meals as 30%, 40%, 30%. According to each patient's required intake, the corresponding nutritional program card is issued, and explained and emphasized at the time of admission and discharge, respectively. Patients could choose different food items on the cards according to their food preferences and actual situation. The experimental group was required to consume LES 1 h before bedtime, while the control group did not consume any food 1 h before bedtime.

Experimental group: patients were given additional meal (total calories 200-275kcal, protein 11.5g-18g, complex carbohydrates 25-55g) 1h before bedtime. the LES regimen was: ① whole protein type enteral nutrition; ② whey protein powder + bread or cookie. The addition of meals was carried out 1h before bedtime. The dietary regimen of the experimental group will be deducted from the corresponding late-evening snacks intake.

(1) Preoperative: ① Patients without nutritional risk: independent intake according to the issued diet program card. ② Patients with nutritional risk: daily oral whole-protein enteral nutrition on the basis of general diet after admission.

(2) Postoperative: start drinking small amount of water on the 1st postoperative day, over to liquid diet after ventilation, and may over to autonomous semi-liquid diet + oral whole protein enteral nutrition during the day + LES program on the 2nd postoperative day. As the patient's voluntary intake meets 60% of the body's needs, the daytime oral enteral nutrition support can be gradually stopped and the LES program can be continued.

3) Discharge: daily intake needs to be on time according to the LES regimen.

Control group: no more food 1h before bedtime.

(1) Preoperative: same as the test group.

(2) Postoperative: start drinking small amount of water on the 1st postoperative day, over to liquid diet after ventilation, and can over to autonomous semi-liquid diet + oral whole protein type enteral nutrition during the day on the 2nd postoperative day. As the patient's voluntary intake meets 60% of the body's needs, enteral nutrition support can be gradually stopped.

(3) Discharge: Doctors judge whether the patients need to continue nutritional support according to their dietary recovery, and if they do, their nutritional supplementation should be completed before 1h

before bedtime.

2. biological sample collection:

Serum samples remaining after testing were collected from patients for metabolomics analysis.

3.Clinical information collection:

(1) General information collection: gender, age, height, weight, BMI, ICG15min retention time, name of surgery, duration of surgery, blood loss, blood transfusion, tumor stage, NRS2002, PG-SGA, GLIM malnutrition assessment (diagnosis) criteria diagnosis;

(2) Nutritional indicators collection: collect nutritional indicators such as serum albumin (ALB), prealbumin (PA), transferrin (TRF), and total serum protein (TP) on the 1st day of the subject's admission to the hospital, on the 6th day of the postoperative period, at 1 month after the operation, at 3 months after the operation, and at 6 months after the operation.

(3) Collection of liver function indexes: Liver function indexes such as alanine aminotransferase (ALT), alanine grass aminotransferase (AST), γ-glutamyltransferase (GGT), cholinesterase (CHE), and total bilirubin (TBIL) were collected on the first day of hospitalization, the sixth postoperative day, the first postoperative month, the third postoperative month and the sixth postoperative month of the subjects.

(4) Clinical outcome collection: to collect clinical outcome indicators of subjects after surgery, such as: postoperative complication rate, recurrence rate, recurrence-free survival, quality of life.

5) Medical cost collection: to collect subjects' total hospitalization cost, cost of anti-infective drugs, cost of albumin during hospitalization.

Data analysis:

Descriptive statistics were performed on the clinical data, and the measures that conformed to normal distribution were expressed as mean ± standard deviation ($\bar{x}\pm s$), and the t-test was used between the groups, and the measures that did not conform to normal distribution were expressed as median (interquartile spacing) [M(IQR)], and the Mann-Whitney U test was used between the groups. Count data were expressed as percentages [n(%)], and the chi-square test was used for between-group comparisons, with $P<0.05$ considered statistically significant. The Kaplan-Meier method in STATA 12.0 was used to calculate the recurrence rate and recurrence-free survival in each group, and the log-rank test was used for intergroup comparison.

4. Case selection

4.1 Number of cases

A total of 106 patients needed to be enrolled.

4.2. Inclusion Criteria

1) Age 18-75 years;

2) Meet the diagnostic criteria of China's "Guidelines for Diagnosis and Treatment of Primary Liver Cancer (2022 Edition)", clinically diagnosed with primary liver cancer, hospitalized with radical hepatectomy as the main surgical treatment, no indication of metastasis of the tumor to extra-hepatic organs in preoperative tests and examinations, no absolute contraindications to surgery, complete resection of the liver tumor in the operation, and hepatocellular carcinoma confirmed by postoperative pathological diagnosis；3. Child-Pugh grades A and B;

4) Preoperative Eastern Cooperative Oncology Group Physical Status Score (ECOG-PS) of 0 to 2;

5) The patient is conscious, has normal verbal communication, and is able to cooperate with the relevant examinations;

6) Fully informed about the study and voluntarily signed an informed consent form.

4.3 Exclusion Criteria

1) Failure to meet selection criteria;

2) Nutritional assessment as cachexia;

3) Presence of contraindications to enteral nutrition (EN) or EN intolerance, such as acute gastrointestinal bleeding, intestinal obstruction. (≥grade 3, National Cancer Institute-Common Terminology Criteria for Adverse Events [NCINCI-CTCAE v 5.0]);

4) Simultaneous combination of malignant tumors in other parts of the body;

5) Combined hepatic encephalopathy or definite infection on admission;

6) Known refractory metabolic diseases (e.g., poorly controlled diabetes mellitus or fasting glucose ≥10 mmol/L, hyperthyroidism, hypothyroidism, metabolic acidosis);

7) Decreased renal function (defined as serum creatinine Cr level ≥176.8 μmol/L);

8) Intravenous or oral nutritional supplements, such as proteins, amino acids, etc., applied within one month prior to admission to the hospital;

9) Patients with severe stress or severe complications such as respiratory failure with severe cardiac,

hepatic, renal and other insufficiencies；

10) Persons with mental and neurological disorders who are unable to cooperate with a physician;

11) Alzheimer's disease, cerebral atrophy, acute stage or sequelae of cerebrovascular disease, cognitive impairment;

12) Previously poor adherence to medication and nutritional counseling;

13) Critically ill and difficult to assess;

14) On the liver transplant waiting list or under consideration for liver transplantation, as such patients may discontinue follow-up before the end of the study;

15) Less than 12 months since last localized treatment (TACE or HAIC or ablative therapy);

16) Other circumstances that the researcher considers inappropriate for participation in the study.

4.4 Exclusion criteria

Enrolled cases but meeting one of the following should be excluded:

1) Intraoperative exploration of the tumor has undergone dissemination or is unresectable;

2) Serious intraoperative or postoperative complications of surgical technique within 24h: bleeding or gastrointestinal leakage leading to shock;

3) Serious cardiac, pulmonary, cerebral or renal vital organ accidents or insufficiency during the operation or within 24h after the operation;

4) Postoperative pathology suggesting non-primary or metastatic hepatocellular carcinoma;

5) Patients in the control group who had more than 7 additional meals before bedtime;

6) Patients in the test group who did not have more than 7 bedtime refills during the follow-up period;

7) Those who voluntarily withdrew.

5. Research Methodology and Technical Approach

5.1 Research Methodology

1) Nutritional Assessment

All patients were screened for nutritional risk using the NRS 2002 scale within 24 h of admission, and a score of ≥3 was considered to be at nutritional risk; patients at nutritional risk were assessed for nutrition using the PG-SGA scale and diagnosed with malnutrition according to the GLIM Malnutrition Assessment (Diagnosis) Criteria.

2) Nutritional support therapy

The target intake for all patients was 25-30 kcal/kg/d. Nutritional supportive therapy was given to patients who were at nutritional risk and could not achieve the target intake. Oral nutritional supplements were preferred, and enteral nutritional supplementation was used in patients who still could not achieve 60% of the target amount. Supplemental parenteral nutrition was considered if patients could not achieve 60% of the target amount for 7 days after admission to the hospital.

3) Interventions

From the total daily intake, patients in the test group were divided into approximately 250kcal for late-evening snacks 1h before bedtime (200-275kcal total, 11.5g-18g protein, 25-55g complex carbohydrates); patients in the control group did not eat any more food 1h before bedtime.

Preparations used:

① Enteral nutrition powder: 450kcal/15.9g/100g;

② Whole protein type enteral nutrition suspension, energy density 1kcal/ml, protein 0.05g/ml, complex carbohydrate 0.126g/ml;

③ Whey protein powder, protein content >80g/100g.

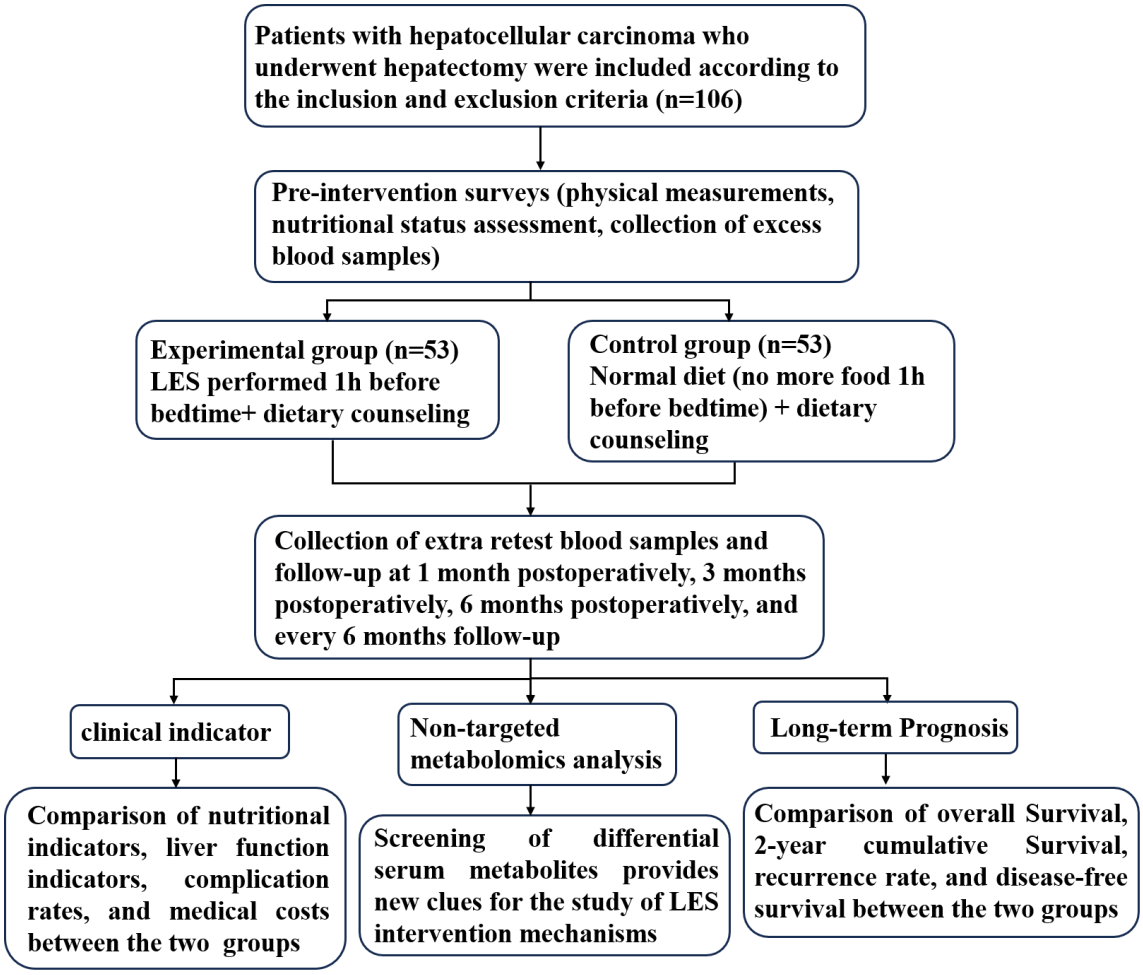
4) Biological sample collection

Post-test residual serum samples from patients on day 1 of admission, day 6 postoperatively, 1 month postoperatively, 3 months postoperatively, and 6 months postoperatively were collected for metabolomic analysis to see if the metabolic pattern of the patients was altered in LES mode.

5) Clinical outcomes

The postoperative complication rate, tumor recurrence rate, recurrence-free survival, postoperative hospitalization time, total hospitalization cost, and cost of anti-infective drugs of the subjects after admission to the hospital were counted, and the chi-square test and independent samples t-test were performed to compare the differences, in order to find out whether the clinical outcomes had been improved under the LES model.

5.2 Technical Approach



6. Observed items and testing time points

6.1 Intake

Daily intake and time of intake were recorded during the perioperative period, and patients were followed up at 1 month postoperatively, 3 months postoperatively, and 6 months postoperatively for intake and time of intake.

6.2 Nutrition indicators

Serum albumin (ALB), prealbumin (PA), total serum protein (TP), and transferrin were collected from subjects on day 1 of admission, day 6 postoperatively, 1 month postoperatively, 3 months postoperatively, and 6 months postoperatively.

6.3 Liver function indicators

The patients' alanine aminotransferase (ALT), alanine transaminase (AST), γ-glutamyl transferase (GGT), cholinesterase (CHE), and total bilirubin (TBIL) were collected on the 1st day of the patients'

admission, on the 6th day of the postoperative period, at 1 month postoperatively, at 3 months postoperatively, and at 6 months postoperatively.

6.4 Medical costs

After the patients were discharged from the hospital, the patients' postoperative length of stay, total hospitalization costs, and drug costs were recorded;

6.5 Clinical outcomes

The time of occurrence of postoperative complications and symptoms of the subjects were recorded daily during the patients' hospitalization, and the recurrence-free survival and quality of survival were followed up at 1 month, 3 months, and 6 months postoperatively.

6.6 Metabolomics testing

Post-test residual serum samples were collected from subjects on day 1 after admission, day 6 postoperatively, 1 month postoperatively, 3 months postoperatively, and 6 months postoperatively for metabolomics testing.

7. Criteria for evaluating the efficacy of treatment

7.1 **Nutritional indicators**

Primary Efficacy Indicator:

Serum albumin (ALB), recording ALB data from patient biochemical tests.

Secondary efficacy indicators:

Prealbumin (PA), alanine aminotransferase (ALT), glutamate aminotransferase (AST), serum gamma-glutamyl transpeptidase (GGT), cholinesterase (CHE), total bilirubin (TBIL), and C-reactive protein (CRP) were recorded, and the corresponding data in the patient's biochemical tests were recorded;

7.2 Clinical outcomes

1) Post-operative complications

a) Postoperative infections: surgical site infections (SSI), remote infections (RI) and bacteremia.

SSI: Shallow/deep incision SSI and organ/space SSI were defined as redness, swelling, heat, pain, and purulent discharge observed in any incision or space operated on intraoperatively, or the need for a new drain within 30 days postoperatively.

Distant infections: including cholangitis, enteritis, pneumonia, catheter-related infections, and urinary tract infections, defined as fever greater than 38.5°C, leukocytosis, and bacteria detected in

sputum, catheter tips, and urine and feces.

Bacteremia: Bacteremia is diagnosed when a strain is cultured from a single blood culture. And when the strain is identified as coagulase-negative staphylococci, it is considered contaminating; coagulase-negative staphylococci may be considered the causative agent when isolated from two or more blood cultures.

b) Bile leakage

Fluid with a bilirubin concentration of at least three times the serum bilirubin concentration drained through an abdominal drain on or after postoperative day 3. Regardless of the initial bilirubin concentration of the drain placed through surgery, the need for radiologic (i.e., interventional drainage) or surgical intervention (i.e., relative to cesarean section) for biliary collection or biliary peritonitis is also part of the diagnosis of bile leakage.

c) Liver failure after hepatectomy

Elevated serum bilirubin concentration and elevated INR on or after postoperative day 5 compared to the previous day; or coagulation factors such as fresh frozen plasma (FFP) are required to maintain normal INR values on or after postoperative day 5 in combination with hyperbilirubinemia.

2) Total hospitalization cost: the total cost of the patient from admission to discharge;
3) Recurrence-free survival: the time from the start of the patient's hepatectomy to the first detection of tumor recurrence;
4) Quality of Survival: Quality of Survival was evaluated by the EORTC QLQ - HCC18 scale (Appendix 1);
5) Safety, diarrhea, vomiting: complaints of gastrointestinal distress that occur after the patient has initiated nutritional support therapy, with diarrhea defined as unformed stools more than three times per day.

8. Adverse Events Watch

Adverse events (AEs), all adverse medical events that occur after a subject receives a late-night snack, which can be manifested as symptoms signs, illnesses, or abnormal laboratory tests, but are not necessarily causally related to the late-night snacks.

Serious Adverse Event (SAE), an adverse medical event such as death, life-threatening, permanent or severe disability or loss of function, subject requiring hospitalization or prolonged hospitalization, and

congenital anomalies or birth defects that occur after a subject receives late-evening snacks.

The investigator will closely observe or follow up the various reactions of the patients after performing the LES in order to detect adverse events or serious adverse events in a timely manner, and all adverse events such as bloating, diarrhea, etc., reported by the subjects or observed by the investigator during the period from enrollment to the end of the study or early withdrawal from the study must be documented in the medical record or CRF, including laboratory and ancillary investigations, and should include, at a minimum, the name of the AE, its occurrence time (to the nearest minute), end time (to the nearest minute), degree and frequency of episodes, whether or not treatment was corrected, the course of treatment and regression if treatment was documented, and the causal relationship to the study. All medical documentation related to the adverse event must be recorded in the original documentation, including copies of requests for laboratory tests and reports of test results. The name of the investigator must be signed and dated (to the nearest minute) on the original documents and written reports.

Changes in the subject's vital signs, physical examination, clinical manifestations, and laboratory tests should be evaluated during the study. At the time of signing the informed consent form, the name and telephone number of an investigator whom the subject can contact in case of an emergency, or report of any medical symptom, or occurrence of an AE related to the subject must be provided. The investigator should follow up all adverse events and serious adverse events, decide the duration of follow-up according to the condition, and give necessary treatment and therapeutic measures during follow-up until proper resolution or stabilization of the condition, and in case of abnormal laboratory tests should be followed up until the return visit is normal to ensure that the damage to the subject is minimized. Detailed records of the follow-up visits and treatment results should be kept.

Toxic reactions or AEs that existed prior to the subject's participation in the clinical study were recorded as AEs only if the grading was found to have increased by one or more grades from the baseline assessment during the study.

CTCAE classification	**equivalent to**	**definition**
Level 1	mildly	Mild discomfort that does not affect daily life or functioning
Level 2	moderately	Unwell, interfering with daily life and function,

		no indication for treatment
Level 3	severe	Symptomatic, affecting daily life and function, with indication for treatment
Level 4	Life threatening/disability/loss of function	Life-threatening conditions, indications for emergency treatment, physically or mentally handicapped conditions
Level 5	death	Adverse events leading to death.

AE and study correlation analysis table

	Definitely	Likely	Possible	Suspicious	unlikely
Reasonable timing with medications/interventions	+	+	+	+	-
Known types of drugs/reactions	+	+	+	+	-
Reduction or disappearance of symptoms with cessation of intervention	+	+	±	±	-
Response recurs after another intervention	+	?	?	?	-
Cannot be explained by disease, co-morbidities	+	+	-	±	-

9. Data security monitoring

A data safety monitoring program will be established for clinical studies according to the level of risk. All adverse events will be recorded in detail, appropriately handled and followed up until properly resolved or stabilized, and serious adverse events and unintended events, etc., will be reported to the Ethics Committee, the competent authority, the sponsor, and the drug regulatory authority in a timely manner according to the regulations; the principal investigator will conduct a cumulative review of all adverse events on a regular basis, and convene a meeting of the investigators to assess the risks and benefits of the study, if necessary; the injuries or discomfort is no more likely and no greater in magnitude than that encountered in daily life or than that of a routine examination, and that data and safety monitoring can be accomplished by the principal investigator.

10. Statistical processing

应用 SPSS26.0 统计软件对临床数据进行描述性统计，符合正态分布的计量资料以均数±标

准差($\bar{x}\pm s$))表示，组间采用 t 检验，不符合正态分布的计量资料以中位数（四分位间距）[M(IQR)]表示，组间采用 Mann-Whitney U 检验。计数资料用百分比[n(%)]表示，组间比较采用卡方检验，P<0.05 为有显著性统计学意义。采用 STATA12.0 中的 Kaplan-Meier 法计算各组复发率及无复发生存期，log-rank 检验进行组间比较。SPSS26.0 statistical software was applied to carry out descriptive statistics of the clinical data, and the measurements that conformed to normal distribution were expressed as mean ± standard deviation ($\bar{x}\pm s$)), and t-test was used between the groups, and the measurements that did not conform to normal distribution were expressed as median (inter-quartile spacing) [M(IQR)], and Mann-Whitney U-test was used between the groups. Count data were expressed as percentages [n(%)], and the chi-square test was used for between-group comparisons, with P<0.05 considered statistically significant. The Kaplan-Meier method in STATA 12.0 was used to calculate the recurrence rate and recurrence-free survival in each group, and the log-rank test was used for between-group comparisons.

11. Ethical principles of clinical research

The clinical study will follow the relevant regulations such as the Declaration of Helsinki of the World Medical Assembly. The clinical study will be implemented only after approval of the trial protocol by the Ethics Committee prior to the commencement of the study. Before each subject is enrolled in the study, it is the responsibility of the investigator to provide the subject or his/her agent with a complete and comprehensive description of the purpose, procedures, and possible risks of the study, and to sign a written informed consent form, which should let subjects know that they have the right to withdraw from the study at any time, and that the informed consent should be retained as a clinical study document for review. Subjects' privacy and data confidentiality will be protected during the study.

12. Reference

[1] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.

[2] Rongshou Zheng, Siwei Zhang, Kexin Sun et al. Analysis of malignant tumor prevalence in China in 2016[J]. Chinese Journal of Oncology, 2023; 45(3):212-220.

[3] Allemani C, Matsuda T, Di Carlo V, et al. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet. 2018;391(10125):1023-1075.

[4] Tsilimigras DI, Bagante F, Sahara K, et al. Prognosis After Resection of Barcelona Clinic Liver Cancer (BCLC) Stage 0, A, and B Hepatocellular Carcinoma: A Comprehensive Assessment of the Current BCLC Classification. Ann Surg Oncol. 2019;26(11):3693-3700.

[5] Finn RS, Ikeda M, Zhu AX, et al. Phase Ib Study of Lenvatinib Plus Pembrolizumab in Patients With Unresectable Hepatocellular Carcinoma. J Clin Oncol. 2020;38(26):2960-2970.

[6] Liu PH, Hsu CY, Hsia CY, et al. Surgical Resection Versus Radiofrequency Ablation for Single Hepatocellular Carcinoma ≤ 2 cm in a Propensity Score Model [published correction appears in Ann Surg. 2016 May;263(5):e77]. Ann Surg. 2016;263(3):538-545.

[7] Jiasheng Yi, Jixiang Zhang, Jing Wang et al. Causes of malnutrition in hepatocellular carcinoma patients and their nutritional treatment[J]. Electronic Journal of Tumor Metabolism and Nutrition,2015;2(03):73-76.

[8] Min Weng, Zhengyan Dai, Kunhua Wang. Nutritional therapy for hepatocellular carcinoma patients in different disease states[J]. Electronic Journal of Tumor Metabolism and Nutrition,2022;9(01):1-6.

[9] Yi Chen, Ling Zeng, Qin Zeng et al. Effects of nutritional supportive therapy on postoperative stress indicators, nutritional status, and long-term survival of hepatocellular carcinoma patients[J]. Chinese Journal of Surgical Oncology,2020;12(03):228-232.

[10] Borhofen SM, Gerner C, Lehmann J, et al. The Royal Free Hospital-Nutritional Prioritizing Tool Is an Independent Predictor of Deterioration of Liver Function and Survival in Cirrhosis. Dig Dis Sci. 2016;61(6):1735-1743.

[11] Huang TH, Hsieh CC, Kuo LM, et al. Malnutrition associated with an increased risk of postoperative complications following hepatectomy in patients with hepatocellular carcinoma. HPB (Oxford). 2019;21(9):1150-1155.

[12] Puri P, Dhiman RK, Taneja S, et al. Nutrition in Chronic Liver Disease: Consensus Statement of the Indian National Association for Study of the Liver. J Clin Exp Hepatol. 2021;11(1):97-143.

[13] Dayu Chen, Xiaojie Bian, Jinchun Chen et al. A study of standardized nutritional drug therapy service model in the perioperative period of liver resection for hepatocellular carcinoma[J]. Pharmacy and Clinical Research,2021;29(03):193-198.

[14] Joliat GR, Kobayashi K, Hasegawa K, et al. Guidelines for Perioperative Care for Liver Surgery: Enhanced Recovery After Surgery (ERAS) Society Recommendations 2022. World J Surg. 2023;47(1):11-34.

[15] Plauth M, Bernal W, Dasarathy S, et al. ESPEN guideline on clinical nutrition in liver disease. Clin Nutr. 2019;38(2):485-521.

[16] Amodio P, Bemeur C, Butterworth R, et al. The nutritional management of hepatic encephalopathy in patients with cirrhosis: International Society for Hepatic Encephalopathy and Nitrogen Metabolism Consensus. Hepatology. 2013;58(1):325-336.

[17] Leoni L, Valoriani F, Barbieri R, et al. Unlocking the Power of Late-Evening Snacks: Practical Ready-to-Prescribe Chart Menu for Patients with Cirrhosis. Nutrients. 2023;15(15):3471.

[18] Guo YJ, Tian ZB, Jiang N, et al. Effects of Late Evening Snack on Cirrhotic Patients: A Systematic Review and Meta-Analysis. Gastroenterol Res Pract. 2018;2018:9189062.

[19] Chen CJ, Wang LC, Kuo HT, et al. Significant effects of late evening snack on liver functions in patients with liver cirrhosis: A meta-analysis of randomized controlled trials. J Gastroenterol Hepatol. 2019;34(7):1143-1152.

[20] Nakaya Y, Okita K, Suzuki K, et al. BCAA-enriched snack improves nutritional state of cirrhosis. Nutrition. 2007;23(2):113-120.

[21] Takeshita S, Ichikawa T, Nakao K, et al. A snack enriched with oral branched-chain amino acids prevents a fall in albumin in patients with liver cirrhosis undergoing chemoembolization for hepatocellular carcinoma. Nutr Res. 2009;29(2):89-93.

[22] Harima Y, Yamasaki T, Hamabe S, et al. Effect of a late evening snack using branched-chain amino acid-enriched nutrients in patients undergoing hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma. Hepatol Res. 2010;40(6):574-584.

[23] Morihara D, Iwata K, Hanano T, et al. Late-evening snack with branched-chain amino acids improves liver function after radiofrequency ablation for hepatocellular carcinoma. Hepatol Res. 2012;42(7):658-667.

[24] Tripodi F, Badone B, Vescovi M, et al. Methionine Supplementation Affects Metabolism and Reduces Tumor Aggressiveness in Liver Cancer Cells. Cells. 2020;9(11):2491.

[25] Rong W, Xia H, Zhang K, et al. Serum metabolic effects of corn oligopeptides with 7-day supplementation on early post-surgery primary liver cancer patients: a double-blind randomized controlled trial. Hepatobiliary Surg Nutr. 2022;11(6):834-847.

[26] Koreeda C, Seki T, Okazaki K, et al. Effects of late evening snack including branched-chain amino acid on the function of hepatic parenchymal cells in patients with liver cirrhosis. Hepatol Res.

2011;41(5):417-422.

[27] Weimann A, Braga M, Carli F, et al. ESPEN practical guideline: Clinical nutrition in surgery. Clin Nutr. 2021;40(7):4745-4761.

[28] Chinese Medical Association Parenteral Enteral Nutrition Section. Guidelines for perioperative nutritional support in adults[J]. Chinese Journal of Surgery,2016;54(9):641-657.

Program signature page

Investigator Statement:

I agree to comply with the review of the Ethics Committee and to start the execution of the clinical trial after approval, to report any changes in the clinical trial activities and unanticipated problems involving risks to the subjects or others to the Ethics Committee in a timely manner, and to execute the study after reapproval of the ethical review. Comply with the requirements of the Ethics Committee for follow-up review and end-of-study review during the study.

I agree to conduct the clinical trial in strict accordance with the design and specifications of the protocol.

I understand that I may interrupt or terminate this clinical trial at any time if it is necessary to ensure the best interests of the subjects.

I agree that I will personally perform or supervise this clinical trial and that I will ensure that all investigators in my organization assisting me in the performance of this clinical trial understand their responsibilities in this clinical trial.

I will strictly adhere to current GCP and the Declaration of Helsinki during the conduct of this clinical trial. I am committed to the moral, ethical and scientific principles of the trial.

During the execution of the clinical trial, I will strictly comply with all laws and regulations related to the clinical trial and protect the rights and interests of patients.

I agree to maintain adequate and accurate medical records and to ensure that these medical records are readily available for audit and inspection in accordance with relevant laws and regulations.

_____	_____	_____
Name (in block letters)	Signature	Date

Appendix 1

EORTC QLQ – HCC18

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

During the past week:	Not at all	A little	Quite a bit	Very much
31. Did you feel thirsty?	1	2	3	4
32. Have you had problems with your sense of taste?	1	2	3	4
33. Have you lost muscle from your arms or legs?	1	2	3	4
34. Have you had abdominal swelling?	1	2	3	4
35. Have you been concerned by the appearance of your abdomen?	1	2	3	4
36. Have you been concerned by your skin or eyes being yellow (jaundiced)?	1	2	3	4
37. Have you had itching?	1	2	3	4
38. Have you had pain in your shoulder?	1	2	3	4
39. Have you had abdominal pain?	1	2	3	4
40. Have you had fevers?	1	2	3	4
41. Have you had chills?	1	2	3	4
42. Have you worried about getting enough nourishment?	1	2	3	4
43. Have you felt full up too quickly after beginning to eat?	1	2	3	4
44. Have you worried about your weight being too low?	1	2	3	4
45. Have you been less active than you would like to be?	1	2	3	4
46. Have you found it difficult to finish things?	1	2	3	4
47. Have you needed to sleep during the day?	1	2	3	4
During the past four weeks:				
48. Has the disease or treatment had any effect on your sex life?	1	2	3	4