北京大学
PEKING UNIVERSITY

Master's Thesis Proposal

A Study on the Interaction between Low-Carbohydrate Diet and Genetic Factors about the Risk of Type 2 Diabetes

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Proposal Date: June 28, 2024

Proposal Location: Public Health Building, Room 418

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1 Research Background and Rationale

1.1 Current Epidemiological Situation of Type 2 Diabetes and Its Risk Factors

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia caused by various factors. The main feature of diabetes is elevated blood glucose levels due to varying degrees of insulin resistance or deficiency. Prolonged chronic hyperglycemia leads to a series of complications involving the cardiovascular system, nervous system, and multiple organ damage, eventually resulting in disability and premature death. It is currently the third primary non-communicable disease that seriously threatens human health, after cardiovascular diseases and cancer. As the economy develops and living standards improve in China, diabetes has become one of the most common chronic diseases that significantly impacts health and daily life. Worldwide, there are currently 537 million people with diabetes, a number projected to rise to approximately 643 million by 2030 and 783 million by 2045. In China, the prevalence of diabetes increased fivefold by 2015, with around 140 million cases and an incidence rate of about 9–12% [1]. Large-scale cross-sectional surveys conducted over the past 30 years in China have shown a continuous upward trend in diabetes prevalence. In 1980, the prevalence rate of diabetes in China was less than 1%. Subsequent national surveys in 1994 and 2000-2001 reported prevalence rates of 2.5% and 5.5%, respectively. The latest national survey in 2007 indicated a prevalence rate of 9.7%. By 2010, the prevalence had risen to 11.6%, and China became one of the countries with a high diabetes prevalence rate (over 10%) [2]. A national survey in 2017 revealed a further increase to 11.2%. These studies indicate that the prevalence of diabetes in China continues to rise without any signs of plateauing or decline. Similarly, the diabetes situation in Beijing is also concerning. Therefore, diabetes has become a significant public health issue in China and globally, and there is an urgent need to identify the reasons behind its high prevalence [3].

Type 2 diabetes mellitus (T2DM) is the most common and fastest-growing type of diabetes, accounting for more than 90% of all diabetes cases. The symptoms of T2DM are often not obvious, making it difficult to detect, and many patients may already have complications by the time they are diagnosed, which increases treatment difficulty and significantly reduces quality of life. T2DM affects a wide range of people, including both the young and the elderly. It is currently understood that T2DM is a multifactorial, progressive, non-communicable chronic disease influenced by both genetic and environmental factors. Environmental risk factors include socioeconomic conditions, personal factors, lifestyle, psychological factors, and environmental pollution. Obesity, hypertension, dyslipidemia, and dietary patterns are common modifiable risk factors [4,5].

In recent years, many cross-sectional studies have shown that over 90% of T2DM patients are overweight or obese, and there is a positive correlation between the prevalence of obesity and diabetes [1,6]. A national health and nutrition survey in the United States found that overweight individuals had a 2.9 times higher risk of developing diabetes compared to individuals with average body weight. From 2010 to 2018, a study by Martina et al. [7] involving 500,000 adults in Spain found that individuals with a BMI ≥ 25 had a 6.74 times higher risk of developing diabetes than those with a BMI < 25. Similarly, a cohort study in Guangzhou by Chen Peiyan et al. [8] revealed that individuals with a BMI > 24 had an odds ratio (OR) of 8.82 for diabetes compared to those with a normal BMI (18.5 < BMI ≤ 23.9). The China Health and Nutrition Survey conducted between 1993 and 2015 found that individuals with a BMI ≥ 24 had a 4.13 times higher risk of developing diabetes compared to those with a BMI < 24 (adjusted OR) [9]. T2DM is also related to the distribution of body fat. Waist-to-hip ratio (WHR) is an essential indicator of central obesity, and individuals with central obesity are more likely to develop T2DM than those with generalized obesity. Cohort studies have shown that WHR is more sensitive than BMI in predicting the risk of T2DM. Therefore, obesity is an independent risk factor for the development of T2DM [10].

Hypertension is another recognized risk factor for T2DM, and there is a shared genetic basis between hypertension and T2DM. The prevalence of hypertension is significantly higher in T2DM patients compared to the general population [11]. A diabetes epidemiological survey conducted in Chongqing from 2012 to 2014 showed that individuals with hypertension had a 3.32 times higher risk of developing T2DM compared to those with normal blood pressure [12]. The "2012-2015 China Hypertension Survey," which involved 451,755 Chinese adults, revealed that the prevalence of hypertension was significantly higher in overweight and obese adults (29.5% and 44.5%, respectively) compared to those with average weight (15.4%, $P < 0.001$) [13]. In Xi'an, Lu Wen conducted a logistic regression analysis of T2DM risk factors and found that hypertension was an independent risk factor for T2DM [14]. T2DM is often accompanied by elevated triglyceride levels and decreased high-density lipoprotein cholesterol (HDL-C), which are manifestations of dyslipidemia. Elevated fasting triglyceride (FTG) levels are considered an independent risk factor for diabetes, as triglycerides compete with glucose to enter cells, leading to insulin resistance. Additionally, elevated triglycerides can interfere with insulin binding to its receptor in peripheral tissues, weakening the effects of insulin. A prospective study by Yang Wenying et al. [39] involving 2,892 non-diabetic individuals found that those with high FTG levels had a 28.1% incidence rate of T2DM after three years, 1.7 times higher than those with low FTG levels [15].

In recent years, China has experienced a rapid nutritional transition characterized by an increase in the consumption of animal foods, dietary fats, and refined grains, alongside a decrease in the consumption of cereals [16]. Between 1991 and 2015, dietary fat intake in China increased from 22% to 34.6%, while animal food intake rose from 38.73% to 64.49%. In contrast, the average daily intake of grains decreased by 142 grams per person, although 60% of total energy intake still came from carbohydrates in 2004 [17,18]. Recent systematic reviews and meta-analyses of cohort studies have shown that maintaining a healthy diet—such as increasing the intake of fiber-rich foods or fish and reducing the intake of red meat, refined grains, and sugary beverages—can lower the risk of developing T2DM [19,20].

1.2 Dietary Factors and Type 2 Diabetes

Diet is a crucial modifiable and preventable factor influencing the burden of type 2 diabetes mellitus (T2DM) and its associated risk factors, playing a critical role in the development of the disease. Numerous epidemiological studies have demonstrated that consuming dairy products, animal fats, and sugars is positively associated with blood glucose levels. At the same time, the intake of cereal fibers, fruits, vegetables, and legumes is negatively correlated with blood glucose levels. Fruits and vegetables are rich in vitamins, minerals, dietary fibers, and other health-promoting phytochemicals. According to the recommendations of the *Chinese Dietary Guidelines*, increasing the intake of fruits and vegetables can reduce the risk of developing diabetes. Additionally, growing evidence suggests that increasing the consumption of oily fish and decreasing the intake of trans fatty acids positively affect cardiovascular health. However, the specific impact of these measures on T2DM remains controversial.

The traditional Chinese diet is primarily based on grains (such as wheat or rice, depending on the geographical location), root vegetables, and legumes, with a high carbohydrate intake. In southern China, steamed rice is the staple food, whereas in northern China, wheat-based foods such as noodles, steamed buns, and dumplings are preferred. A high carbohydrate intake (mainly from refined grains) has been linked to an increased risk of T2DM among Chinese adults. A meta-analysis of prospective studies revealed that daily consumption of 200–400 grams of refined grains is associated with an increased risk of type 2 diabetes, raising the risk by 6–4%. According to a 2020 meta-analysis, 89.7% of carbohydrate intake among the Chinese population comes from white rice, with 35.7% of the population consuming other grains. High carbohydrate intake (mainly from refined grains) is associated with an increased risk of T2DM in Chinese adults.

Moreover, other dietary characteristics may exacerbate common T2DM comorbidities. The sodium content in the Chinese diet is also high, with over 90% of respondents exceeding the recommended sodium intake (<2000 mg/day). Between 1992 and 2012,

refined grain consumption decreased by 26%, and whole grains and vegetable consumption declined. However, during the same period, sugary beverages, dietary fats, red meat, and processed grains increased steadily. The excessive consumption of these high-fat, high-sugar, and high-calorie foods puts consumers at higher risk for T2DM and other metabolic diseases. Among the various dietary risk factors, changes in daily carbohydrate intake (including refined rice and sugary drinks) and increased intake of unhealthy fats are likely to have the most significant impact, as carbohydrates and fats are common in the diet.

Compared to the health effects of individual foods and nutrients, a holistic view that considers the overall dietary pattern, including the intake of various foods and nutrients, allows for a deeper exploration of their complex interactions and helps to analyze their relationship with overall health outcomes. This comprehensive approach forms an essential basis for scientifically supported nutritional interventions and health education. As a result, this study area has gained significant attention in nutritional epidemiology and has been emphasized in dietary guidelines in multiple countries.

Previous research has identified several dietary patterns that can prevent and manage T2DM, including low-fat diets (LFD), low-carbohydrate diets (LCD), Mediterranean diets (MedDiet), and vegan diets. However, LCD and MedDiet originate from Western countries, and the availability of foods, cultural differences, religious beliefs, health awareness, and traditional customs challenge the adoption of these dietary patterns in China. In particular, the promotion of vegan and vegetarian diets is somewhat limited, as they exclude entirely animal-based foods.

1.3 Low-Carbohydrate Diet Pattern and Type 2 Diabetes

Carbohydrates (CHO) are the only macronutrients directly increasing blood glucose levels. They can stimulate insulin secretion directly or influence insulin release through the indirect action of insulin and other hormones [34]. When assessing the health impacts, it is essential to consider the balance and types of macronutrients or the overall diet rather than focusing solely on a single nutrient [35,36]. A low-carbohydrate diet (LCD), characterized by reduced carbohydrate intake and relatively increased proportions of protein and fat, has been recommended in many reviews as an initial treatment for diabetes [37]. In recent years, LCD has been advocated as the primary dietary intervention in nutritional epidemiology, with studies on its association with T2DM and its risk factors increasing more than fivefold [31,38].

Although there is some debate, studies suggest that LCD may help reduce T2DM and its associated risk factors due to several mechanisms: First, when daily CHO intake is low, the body cannot fully utilize glucose as an energy source and shifts to using stored energy from fat tissue, inhibiting hepatic gluconeogenesis and improving lipid metabolism by producing ketone bodies. These ketone bodies can be oxidized in the

liver for energy or excreted through urine, and this process helps burn fat and aids in weight loss, providing multiple benefits for T2DM patients [31,39]. Second, LCD has favorable effects on weight and lipids (such as high-density lipoprotein (HDL), cholesterol, and triglycerides) while not affecting low-density lipoprotein (LDL) and glucose metabolism. This decreases blood pressure, serum cholesterol, and glucose levels, slowing the progression of hypertension, dyslipidemia, and diabetes, effectively reducing the risk of T2DM [11,37,40,41]. Third, studies have found that CHO intake is associated with an increased risk of T2DM, whereas protein and fat intake are not [42]. Therefore, compared to other dietary patterns, when LCD reduces the CHO ratio to 10-20% and increases the protein or fat ratio to 50% and 44.3-54.7%, respectively [43], the satiety provided by protein and its ability to suppress hunger have shown sound short-term effects [44,45]. Regarding compliance, LCD has significant advantages due to its dietary characteristics, making it easier for individuals to adhere to the regimen [41]. In 2018, the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) stated that individuals could choose LCD based on their needs and preferences.

However, there are several reasons why LCD has yet to be widely adopted. First, fiber-rich foods (such as whole grains and fruits) intake is lower. In comparison, animal fats, proteins, and saturated fatty acids intake are higher, leading to potential nutritional imbalances in LCD [3,46]. Second, in China, the daily dietary structure relies heavily on grains and starches, which form a large portion of carbohydrate intake. The low CHO content of LCD might reduce palatability, making it difficult for individuals to adhere to the diet long-term and making it less acceptable for the Chinese population [47]. Third, high fat and protein intake can cause digestive discomfort, bad breath, constipation, an increased risk of gallbladder disease, and pain due to rapid weight loss [43].

Considering both the advantages and limitations of LCD, Halton et al. [48] 2006 established a Low-Carbohydrate Diet Score (LCDS) based on the relative levels of fat, protein, and carbohydrate intake. The LCDS reflects the dependency on carbohydrates, with the benefit of gradually reducing carbohydrate intake while relatively increasing fat and protein intake. The total LCDS can be calculated along with scores for plant-based and animal-based LCD. Based on a prospective epidemiological cohort study involving 135,335 participants from 18 low-, middle-, and high-income countries, the results showed no adverse association between T2DM and LCD. During a 10-year follow-up, only high-carbohydrate diets (>70% of energy intake) were associated with increased cardiovascular and all-cause mortality [49]. The quality of the macronutrient replacing carbohydrates is also a key consideration—replacing carbohydrates with animal fats or proteins is associated with increased mortality while replacing them with plant-based unsaturated fats and proteins is associated with reduced mortality [50]. The

source of carbohydrates is also essential; when assessing the relationship between carbohydrate intake and T2DM outcomes, the quality of carbohydrates may be more important than the quantity.

Research on LCD and T2DM, as well as its risk factors, remains controversial. Some studies have found a negative association between LCD and T2DM risk [43,45,52,53], while others found no significant association [48]. The Japan Public Health Center-based prospective study observed a reduced risk of T2DM related to LCDS in women but not men [54–56]. A U.S. prospective cohort study found no significant association between high scores for plant-based protein and fat and T2DM risk [57]. Previous research on T2DM and the risk factors of LCD has yielded conflicting results, suggesting that LCD is negatively associated with weight, BMI [19,24,48,55,58], blood glucose [5,48,55], total cholesterol, and LDL [54,55], or unrelated to cardiovascular disease [37,59–61].

1.4 Gene-Diet Interaction in Type 2 Diabetes

In previous studies, over 1,700 [79,80] susceptibility loci for type 2 diabetes mellitus (T2DM) have been reported through genome-wide association studies (GWAS), and more than 400 of these loci have been successfully identified and replicated. These include genes such as transcription factor 7-like 2 (TCF7L2) rs12255372, fat mass and obesity-associated protein (FTO) rs8050136, peptidase D (PEPD) rs3786897, and apolipoprotein E (APOE) rs7412 [38,81–86]—the development of T2DM results from interactions between genes and between genes and the environment. TCF7L2 and FTO are major transcription factors (TF) in the Wnt signaling pathway, regulating glucose homeostasis in the pancreas and playing a crucial role in glucose-stimulated insulin secretion (GSIS) and the survival of pancreatic β-cells [87,88]. Most genes that determine susceptibility to glucose and lipid metabolic disorders regulate insulin secretion, sensitivity, and pancreatic β-cell function.

In early 2006, Grant et al. [89] first reported a significant association between the TCF7L2 gene polymorphism and T2DM. Since then, the relationship between TCF7L2 polymorphisms and T2DM has received worldwide attention. It has been verified in various populations, leading to relatively consistent conclusions: TCF7L2 is the gene most widely confirmed to be associated with T2DM across different populations, making it one of the most common T2DM susceptibility genes globally. In the initial study by Grant et al. [89], rs7903146 was identified as the most critical risk variant for T2DM. Almost all subsequent European and United States studies confirmed the significant association between rs7903146 and T2DM, with reports from Asian and Arab populations as well. The intron variant rs7903146 (C > T) is substantial, increasing the risk of T2DM threefold in Arab patients. A recent meta-analysis demonstrated that rs7903146 is significantly associated with T2DM in Caucasians, East Asians, South Asians, and other ethnic groups [90]. TCF7L2 regulates glucose

metabolism in the liver and pancreas [91] and regulates fat synthesis signaling [92]. Research suggests that genetic variation in TCF7L2 may lead to dysfunctional fat cells, potentially contributing to T2DM [93].

FTO is the first candidate obesity gene identified in humans. It is widely expressed in human tissues, with the highest expression in the brain, particularly in the hypothalamus. The relationship between the FTO gene and factors such as food intake, food preferences, physical activity, and basal metabolic rate has been studied. Still, conclusive results have yet to be reached [94]. However, FTO plays a role in regulating energy homeostasis by influencing DNA repair and modification processes. Studies have shown that rs8050136 within the FTO gene is significantly associated with a higher risk of T2DM in Asian and European populations [87,95,96]. Furthermore, several meta-analyses confirmed the significant association between rs8050136 and an increased risk of T2DM [97–102].

Recent research suggests that n-3 polyunsaturated fatty acids (PUFAs) have the potential to interact with various candidate gene variants to regulate the risk of chronic diseases such as cardiovascular diseases, metabolic syndrome, and T2DM [103]. The PEPD gene encodes a member of the peptidase D family, which plays a crucial role in proline metabolism and collagen degradation. In diabetic patients, increased PEPD activity enhances collagen breakdown [104]. According to a GWAS meta-analysis, PEPD rs3786897 is associated with T2DM risk, particularly in East Asian populations [105,106]. Zheng et al. [107] found that the PEPD rs3786897 variant interacts with red blood cell n-3 fatty acids to regulate T2DM risk in the Chinese population, and higher n-3 fatty acid levels can neutralize the genetic risk of the rs3786897 A allele [107].

The APOE gene, which plays a critical role in lipid and lipoprotein transport, is considered the most potent genetic determinant of variation in low-density lipoprotein cholesterol (LDL-C) and is an independent risk factor for T2DM and obesity [108]. Studies have shown a significant association between APOE polymorphisms and lipid concentrations, which is common among Chinese T2DM patients. The APOE2 allele, determined by rs7412, weakly binds to LDL receptors, increasing receptor activity and reducing plasma LDL levels [109]. A cross-sectional study in Brazil found that the rs7412 variant significantly reduces the risk of dyslipidemia, consistent with previous studies, which reported that carriers of the rs7412-CT/TT genotype had a significantly lower risk of dyslipidemia [110].

With advances in biological technologies and genetic epidemiology, many potentially associated with T2DM genes have emerged. However, several key issues remain. First is the uncertainty of the relationship between genes and T2DM: it is still unclear which specific genes determine T2DM, and these loci roles in T2DM development remain ambiguous [11]. Second, the limitations of GWAS in determining

disease susceptibility: using GWAS to identify genomic variations that help assess disease susceptibility is still a challenge [111]. Third, challenges in early prevention and intervention: while screening for susceptibility genes may allow for early prevention or delay of T2DM onset to some extent, implementing genetic screening and effective early interventions faces technical, ethical, and social challenges.

Significance of this study:

1. Early detection of high-risk populations: Screening for specific susceptibility genes can help identify high-risk populations early.
2. Adjusting environmental factors: Simultaneously modifying ecological risk factors, such as changing dietary behavior in high-risk populations, can help achieve early prevention or delay the onset of diabetes.
3. Providing a theoretical basis for gene therapy and pre-disease diagnosis: This study provides a solid theoretical foundation for future gene therapy, screening, and pre-disease diagnosis.

2 Research Objectives

2.1 To explore the relationship between a low-carbohydrate diet and type 2 diabetes and to further assess the quality of carbohydrates while considering their health effects.

2.2 To investigate the interaction between the intake of different food sources and significant susceptibility gene loci for type 2 diabetes and their impact on the disease.

3 Research Content

3.1 Type 2 Diabetes and Health Effects of Low-Carbohydrate Diets and Carbohydrates

Using data from the 2005-2021 cohort study on common chronic non-communicable diseases among residents in rural northern China, dietary assessments and low-carbohydrate diet (LCD) pattern evaluations were conducted to analyze the relationship between type 2 diabetes and the health effects of a low-carbohydrate diet and carbohydrate intake.

3.2 Relationship between Low-Carbohydrate Diets, Genetic Loci, and Type 2 Diabetes

The interaction between genetic loci and a low-carbohydrate diet was analyzed using a generalized estimating equation model embedded in a family structure. The model was adjusted for age, gender, and lifestyle, and the multiplicative interaction term between the low-carbohydrate diet and genetic loci was introduced. The significance of the interaction between these factors was

determined by evaluating the P-value of the interaction term.

4. Subjects and Methods

4.1 Study Design and Subjects

This study is a population-based prospective cohort study, with data from the "Family Cohort Study on Common Chronic Non-Communicable Diseases in Northern Rural Areas" project [112]. The project selected administrative villages from nine townships in Fangshan District, Beijing, which have typical demographic, economic, and geographical characteristics of northern regions, as research sites. Participants were recruited by family units, with baseline data collected from June 2005 to August 2017. The first follow-up survey was completed from July 2019 to December 2021, with 3,156 participants included after applying the inclusion and exclusion criteria.

The questionnaire survey covered demographic characteristics (such as age, gender, and educational background), family medical history, medication history, medical consultation history (including age at first onset, disease diagnosis, and treatment details), and environmental and lifestyle risk factors (such as smoking, alcohol consumption, physical exercise, etc.). The clinical physical examination included measurements such as height, weight, waist circumference, and hip circumference, and the biochemical tests measured indicators such as blood pressure, blood glucose, blood lipids, and inflammatory cytokines. Gene polymorphism testing involves extracting DNA to detect single nucleotide polymorphisms (SNPs). Follow-up work included annual clinical examinations and registration of new diabetes cases. Behavioral risk factors and diseases were reassessed every two years.

The inclusion criteria for participants were as follows: (1) individuals aged ≥18 years at the time of cohort entry; (2) completion of a full dietary FFQ (Food Frequency Questionnaire) survey; (3) complete SNP testing data; (4) voluntary participation with completion of the questionnaire survey, physical examination, and biochemical testing; and (5) provision of informed consent and willingness to cooperate with the study. The exclusion criteria included individuals with significant or severe chronic diseases, such as malignant tumors or serious liver and kidney diseases that would prevent participation in the survey. This study was approved by the Peking University Biomedical Ethics Committee (Approval No.: IRB00001052-13027), and all participants provided written informed consent.

4.2 Research Methods

4.2.1 Questionnaire Survey

This study's questionnaire survey incorporates real-time logic checks and is administered by trained and experienced personnel. The questionnaire primarily covers general demographic information (such as gender, age, marital status, education level, household income, etc.), lifestyle behaviors (such as smoking, alcohol consumption, physical activity, and intake of fruits and vegetables), medical history (including diabetes, hypertension, and dyslipidemia), medication history (for hypertension, diabetes, and dyslipidemia), treatment history, and female menstrual and reproductive history. Smoking is defined as currently or previously smoking at least one cigarette per day (or using other forms of tobacco equivalent to one cigarette) for at least one year. Alcohol consumption is defined as currently or previously drinking at least once a week for at least one year. Regular physical activity is defined as engaging in physical labor or exercise at least three times per week, with each session lasting at least 30 minutes.

4.2.2 Clinical Biochemical and Physical Examination

The measurements, conducted by professionally trained personnel, include height, body weight, waist circumference (WC), hip circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP). Height and body weight are measured using a standard height-weight scale, and body fat percentage is calculated using the Japanese TANITA TBF-418B professional body fat analyzer, employing bioelectrical impedance analysis. Height is recorded to an accuracy of 0.1 cm, and body weight to an accuracy of 0.1 kg.

Blood pressure measurements: Before measuring, subjects are asked to rest in a calm environment for 10 minutes to avoid emotional fluctuations. In a sitting position, blood pressure is measured three times on the left arm using an Omron HEM-7200 electronic blood pressure monitor, with 1-minute intervals between measurements. The first reading is discarded, and the average of the second and third readings is taken as the final blood pressure value.

Biochemical tests include fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). Table 1 lists detailed indicators. Other studies on this research population describe specific measurement methods [113].

Table 1: Clinical Biochemical and Physical Indicator

Indicator	Content
FPG，mmol / L	< 5.6 or medication use
TC，mmol/L	< 5.2
TG，mmol / L	< 1.70
LDL-C，mmol / L	0~3.1
HDL-C，mmol/L	0.9~2
BP，mmHg	90 < SBP < 120 or 60< DBP < 80

4.2.3 Dietary Assessment

This study used a self-designed semi-quantitative Food Frequency Questionnaire (FFQ). Based on the dietary characteristics of the target population, the questionnaire was adapted from the simplified Chinese version of the Food Frequency Questionnaire for the Chinese population, aiming to assess the participants' past dietary habits. The food items in the questionnaire included grains and tubers, legumes, vegetables and fruits, fish, poultry, eggs, dairy products, legumes and nuts, dietary supplements, oils (both animal and plant-based), alcohol, tea, seasonings, and beverages, totaling 23 food categories. The frequency of consumption was categorized from low to high, with none occurring 1-3 times per week, 4-6 times per week, daily, and the average portion size per consumption. Participants were required to select their weekly consumption frequency for each food item, and the average daily intake frequency for each food category was calculated based on the responses. Details are shown in Table 2.

Table 2: Food Groups and Food Types in the Food Frequency Questionnaire

Food Group	Food Types
Grains	Rice
Flour-based foods	Steamed buns, noodles, flatbread, etc.
Root vegetables	Potatoes, sweet potatoes, taro, cassava
Fried foods	Fried dough twists, spring rolls, fried meatballs, deep-fried dough sticks, fried cakes, fried buns, French fries, fried bread, etc.
Beans and bean products	Tofu, soft tofu, soy milk, bean products, soybeans, etc.
Fresh vegetables	Spinach, Chinese cabbage, cucumber, tomatoes, radish, etc.
Fresh fruits	Bananas, watermelon, Hami melon, etc.
Meat	Pork, beef, lamb, chicken, duck, ham, etc.
Processed meats	Bacon, sausage, ham, smoked sausages, etc.
Animal organs	Animal organs such as liver, kidneys, intestines,

	small intestines, heart, etc.
Aquatic products	Freshwater fish, seawater fish, shrimp, shellfish, crabs, etc.
Dairy products	Whole milk, low-fat milk, whole milk powder, skim milk, etc.
Eggs	Chicken eggs, duck eggs, quail eggs, etc.
Edible oils	Peanut oil, soybean oil, rapeseed oil, etc.
Salt	Salt
Alcoholic beverages	Low-alcohol spirits, high-alcohol spirits, beer, fruit wine, etc.
Tea	Green tea, black tea, etc.
Nuts	Dried nuts
Sweets	Biscuits, bread, etc.
Sugary drinks	Common milk tea, carbonated beverages, sports drinks, fruit juices, etc.

4.2.4 Evaluation of the Low-Carbohydrate Diet (LCD) Pattern

The adherence of study participants to the low-carbohydrate diet pattern is assessed using the Low-Carbohydrate Diet Score (LCDs). The scoring of food items follows the method reported in the literature for LCDs [114].

Based on the "Chinese Food Composition Table," the average daily intake of foods is calculated by combining the frequency of consumption and the average portion size per intake. The total energy and macronutrient energy ratios for all participants are then calculated, which include total carbohydrates, total fat, total protein, plant fat, plant protein, animal fat, and animal protein. The formula for calculating the macronutrient energy ratio is:

The energy provided by the nutrient/Total energy×100%

The macronutrient energy ratios are divided into 11 levels, each assigned a score. In the total LCD score, points are assigned for total carbohydrate, fat, and protein. The energy ratio for each macronutrient is scored out of 10. For carbohydrates, scores range from 10 (lowest intake) to 0 (highest intake) and are assigned negatively, while for protein and fat, scores are assigned positively, ranging from 0 (lowest intake) to 10 (highest intake). The scores for each macronutrient are summed to obtain the total LCD adherence score. The higher the total LCD score, the lower the carbohydrate intake and the higher the fat and protein intake.

For the plant-based LCD score, the total score is calculated by summing the scores for total carbohydrates, plant fat, and plant protein, where carbohydrates are scored negatively, and plant fat and protein are scored positively. A higher plant-based LCD score indicates lower carbohydrate intake and higher intake of plant fat and protein,

reflecting greater adherence to the plant-based low-carbohydrate diet. For the animal-based LCD score, the total score is calculated by summing the total carbohydrates, animal fat, and animal protein, where carbohydrates are scored negatively, and animal fat and protein are scored positively. A higher animal-based LCD score indicates lower carbohydrate intake and higher intake of animal fat and protein, reflecting greater adherence to the animal-based low-carbohydrate diet. The adherence scores for the three types of LCDs (total, plant-based, and animal-based) range from 0 to 30. The higher the score, the greater the adherence to the low-carbohydrate diet. The specific scoring method is shown in Table 3.

Table 3: LCD Scoring Standards

Score	Total carbohydrates	Total protein	Total fat	Plant protein	Plant fat	Animal protein	Animal fat
0	>71.5	< 8.6	< 18.9	< 6.1	< 10.2	< 1.1	< 3.4
1	67.6-71.5	8.6-9.4	18.9-22.4	6.1-6.7	10.2-12.8	1.1-1.5	3.4-4.8
2	64.8-67.5	9.5-9.9	22.5-25	6.8-7.1	12.9-14.7	1.6-1.9	4.9-6.2
3	62.5-64.7	10.0-10.4	25.1-27.4	7.2-7.5	14.8-16.6	2.0-2.2	6.3-7.5
4	60.2-62.4	10.5-10.9	27.5-29.6	7.6-7.8	16.7-18.3	2.3-2.7	7.6-8.8
5	57.9-60.1	11.0-11.3	29.7-31.8	7.9-8.1	18.4-20.1	2.8-3.1	8.9-10.2
6	55.5-57.8	11.4-11.8	31.9-34.4	8.2-8.4	20.2-22.2	3.2-3.5	10.3-11.7
7	52.7-55.4	11.9-12.5	34.5-37.3	8.5-8.7	22.3-24.9	3.6-4.0	11.8-13.4
8	49.5-52.6	12.6-13.2	37.4-40.9	8.8-9.1	25.0-28.2	4.1-4.7	13.5-15.7
9	44.0-49.4	13.3-14.3	41.0-46.3	9.2-9.8	28.3-34.1	4.8-5.9	15.8-20.0
10	< 44.0	> 14.3	> 46.3	> 9.8	> 34.1	> 5.9	> 20.0

4.2.5 Gene Polymorphism Testing

This study collected venous blood samples from the subjects, and leukocyte DNA was extracted from blood clots using the phenol-chloroform method according to standard procedures for gene polymorphism testing. A qualified testing company was entrusted to perform the genotyping analysis. First, polymerase chain reaction (PCR) was used to amplify the genes in a high-throughput water bath system (Hydrocycler). Next, LGC's competitive allele-specific PCR (KASP) technology was applied for high-throughput genotyping analysis. The steps for gene polymorphism testing include:

1. We are designing primers for the selected gene loci.
2. We are synthesizing the primers.
3. I am performing the PCR amplification reaction.
4. Amplification is continued until the genotyping is complete, using the BMG PHERAstar instrument to detect fluorescence signals and analyze the genotyping results.

5. We are exporting the genotyping results.

The concentration of DNA was measured using ultraviolet spectrophotometry. The steps include taking 2.0 μL of DNA, adding 118 μL of ultrapure water to achieve a 60-fold dilution, and then using a UV7501 ultraviolet spectrophotometer to measure the absorbance at 260 nm and 280 nm.

The formula to calculate DNA concentration is:

$$DNA\ concentration\ (\mu g/ml) = (OD260 \times 50 \times dilution\ factor \div 1000) \div 1000$$

OD260 represents the absorbance at 260 nm, the dilution factor is 60, and 50 indicates that when the absorbance of double-stranded DNA at 260 nm is 1, the concentration is 50 μg/ml.

The DNA purity can be calculated by the ratio of OD260 to OD280. Ideally, the purity value should be between 1.8 and 2.0. A value lower than 1.8 may indicate contamination by proteins or phenol, while a value higher than 2.0 may suggest RNA contamination.

4.3 Data Sorting and Analysis

4.3.1 Descriptive Analysis

Descriptive statistical analysis was conducted using SPSS 26.0 software to analyze the general demographic characteristics of T2DM patients and non-patients. The mean ± standard deviation (SD) was used for continuous variables to express the data. For normally distributed variables, a t-test was employed to compare differences between groups. The median (P25, P75) was reported for non-normally distributed variables, and the Wilcoxon rank-sum test was used to compare differences between groups. Categorical variables were presented as frequencies (percentages). The chi-square test was used for unordered categorical variables, and the chi-square trend test was applied for ordinal categorical variables.

4.3.2 Dietary Analysis

The intake of dietary carbohydrates is expressed as a percentage of total energy using the nutrient density method and is divided into quartiles (<49%, 49-56%, 56-63%, and≧63% of energy from carbohydrate intake). For continuous variables, the overall characteristics are presented as mean ± standard deviation (SD), and for categorical variables, they are presented as proportions.

4.3.3 Gene-Environment Interaction Analysis

The analysis of gene-environment interactions was conducted using the R 4.2.2 software, with family coding used as the grouping variable. A generalized estimating equation (GEE) model, embedded with family structure, was applied to analyze the interaction between genetic loci and a low-carbohydrate diet. The model adjusted for

age, gender, lifestyle, and education level. An interaction term between the low-carbohydrate diet and genetic loci was introduced, and the P-value of the interaction term was used to determine whether there was an interaction between the factors. The Bonferroni correction was applied for multiple comparisons to avoid false positive results. The study included 4 genetic loci, so the significance threshold for hypothesis testing was set at 1.11×10^{-3}.

4.4 Potential Bias and Quality Control

4.4.1 Selection of Investigators: Investigators must have a rigorous work ethic, a scientific attitude, and the professional knowledge necessary to conduct the investigation.

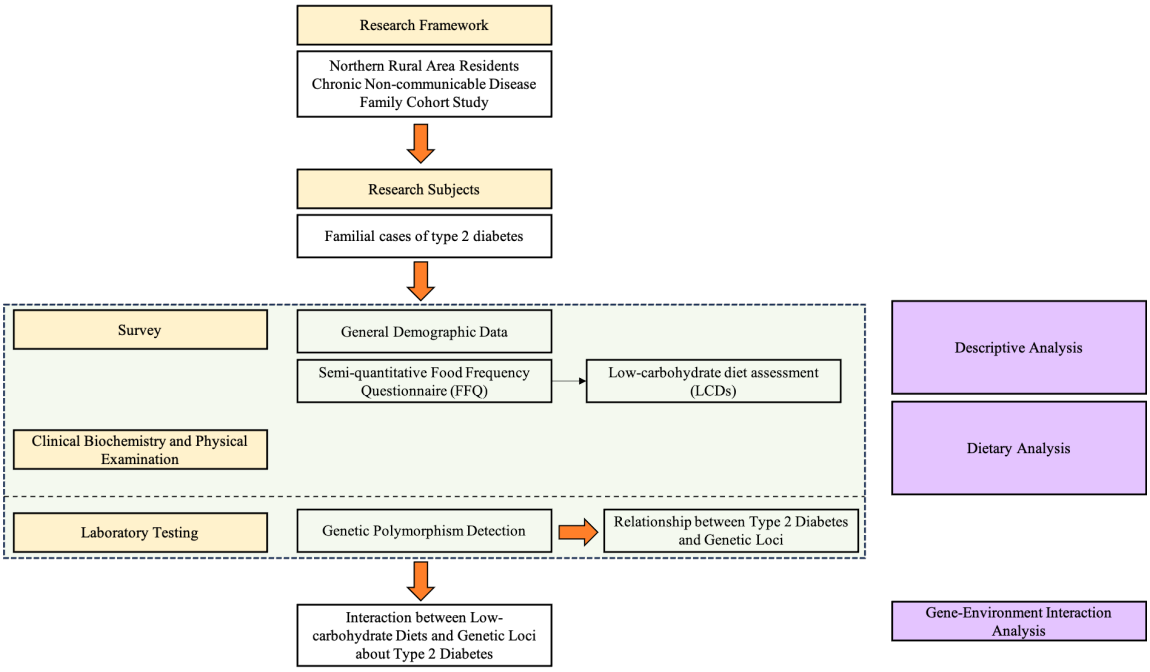
4.4.2 Investigator Training: Core Peking University research team members provided uniform and strict training to all investigators. They ensured mastery of standardized methods and techniques and evaluated the investigators before the survey began.

4.4.3 On-Site Survey: Quality control personnel verified the completeness of all questionnaires and physical examination forms, with a 1% sample checked for completeness and accuracy. Epidata 3.0 software was used for double entry in parallel during data entry, with logical checks applied.

4.4.4 Supervision: In the follow-up and confirmation of outcome events, monitoring systems, telephone follow-ups, and on-site visits were used. Additionally, more than 99% of the population was covered by the health insurance system, effectively reducing the underestimation or omission of outcome events. Verification through hospital databases reduced the risk of overestimating outcome events.

4.4.5 Biochemical Testing: The biochemical testing adhered to the quality evaluation standards set by the Beijing Clinical Testing Center, with strict quality control measures implemented within and between laboratories.

5. Technical Roadmap



6. Expected Results

6.1 The relationship between Type 2 Diabetes and Low-Carbohydrate Diet (LCD), and the association between carbohydrate intake and type in LCD with Type 2 Diabetes.

6.2 The impact of the interaction between different food intake and major susceptibility genes for Type 2 Diabetes on the development of Type 2 Diabetes.

7. Feasibility Analysis

This research project is based on a large cohort study titled “Family Cohort Study of Common Chronic Non-Communicable Diseases in the Rural Population of Northern China.” The National Natural Science Foundation supports the project and collaborates with community health service institutions in Fangshan District, Beijing, including township hospitals, community health service centers, and village clinics, which provide support in terms of personnel, funding, and research sites. The project plans to recruit 1,000 stroke patients, 2,000 hypertension patients, and 2,000 individuals for the cohort study.

During my literature review, I systematically studied the theories related to nutritional epidemiology, family studies, and cohort studies. Through in-depth research, I gained a clearer understanding of the theoretical framework in this research field. At the same time, by conducting field visits, I accumulated valuable practical experience and deepened my knowledge of the research topic. This work series has enhanced my professional skills and laid a solid foundation for subsequent research. I have already

obtained a systematic set of preliminary data, which will be crucial for supporting my research.

In the next phase, I will focus on conducting gene polymorphism analysis. By systematically collecting relevant literature, I will explore the interaction between genes and environmental factors, focusing on the interaction between genes and diet. This research direction will help to more comprehensively understand related health issues and provide valuable scientific support for the development of the field of public health. Through in-depth research on gene-environment interactions, I hope to give a scientific basis for preventing and controlling related diseases, contributing more deeply to human health.

In future research, I will continue to work hard to refine my theoretical system, expand the depth of my research, and make more significant contributions to academia and public health.

My supervisor has discussed and approved the technical roadmap for the research work. (1) I have the guidance of my supervisor in theoretical research and organizing the logic of the thesis; (2) relevant reference books can be borrowed from the library; (3) I can access a large amount of related literature through the data resources of Peking University Library, which provides theoretical solid support; (4) reliable field investigations strongly support the research process and conclusions.

8. Research Timeline

July 2023 – September 2023: Review relevant literature and establish key research points.
October 2023 – December 2023: Finalize the thesis topic.
January 2024 – April 2024: Collect relevant data.
May 2024 – September 2024: Organize and analyze the data.
October 2024 – March 2025: Write the thesis.
March 2025 – April 2025: Finalize the thesis draft.

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