The Dietary Branched-Chain Amino Acids Transition and Risk of Type 2 Diabetes Among Chinese Adults From 1997 to 2015: Based on Seven Cross-Sectional Studies and a Prospective Cohort Study

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Background: The situation is grim for the prevention and control of type 2 diabetes (T2D) and prediabetes in China. Serum and dietary branched-chain amino acids (BCAAs) were risk factors for T2D. However, there is a lack of information on trends in consumption of BCAAs and the risk of T2D associated with BCAAs intake, based on nationally representative data in China. Thus, we aimed to comprehensively describe the dietary BCAAs transition and risk of T2D, at a national level among Chinese adults from 1997 to 2015.

Methods: The data sources were the China Health and Nutrition Survey (CHNS) and China Nutrition and Health Survey (CNHS). Cross-sectional data on intake were obtained from CHNS (1997, n = 9,404), CHNS (2000, n = 10,291), CHNS (2004, n = 9,682), CHNS (2006, n = 9,553), CHNS (2009, n = 9,811), CHNS (2011, n = 12,686) and CNHS (2015, n = 71,695). Prospective cohort data were obtained CHNS (1997–2015, n = 15,508).

Results: From 1997 to 2015, there was a significant decreasing trend in the BCAAs intake of Chinese adults in all subgroups (P < 0.0001) except for Leu in 80 or older, and a decreasing trend in the consumption of BCAAs after 40 years old (P < 0.05). The mean intake of BCAAs in the population of cohort study was 11.83 ± 3.77g/day. The 95% CI was above the HR of 1.0, when the consumptions were higher than 14.01, 3.75, 6.07, 4.21 g/day in BCAAs, Ile, Leu and Val, based on RCS curves. According to the Cox proportional hazards models, Compared with individuals with BCAAs consumption of 10.65–12.37 g/day, the multivariable-adjusted HR for diabetes was 2.26 (95% CI 1.45 to 3.51) for individuals with consumption of BCAAs more than 18.52 g/day. A statistically significant positive association between BCAAs intake and risk of T2D was observed in males or participants aged 45 years and older, but not in females or participants younger than 45 years.
Conclusion: Our results reveal a trend toward decreased BCAAs intake in Chinese from 1997 to 2015. After 40 years of age, consumption of BCAAs declined with increasing age. Higher BCAAs intake was associated with higher risk of T2D. This relationship is more stable among men and middle-aged and elderly people.

Keywords: nutritional epidemiology, branched chain amino acids, transition, nutrient effects, type 2 diabetes, risk analysis

INTRODUCTION

Branched-chain amino acids (BCAAs), including leucine (Leu), isoleucine (Ile), and valine (Val), are essential amino acids for mammals (1) and are supplied considerably from diet. Previous studies have shown that the main food sources of BCAAs in the US population were meat (37%), milk (12%), and fish (8%), while in the Japanese population the main contributors were cereals, potatoes and starches (23–25%), fish and shellfish (21–23%) and meat (14–15%) (2). BCAAs were critical components of dietary protein. Elevations in branched-chain amino acids (BCAAs) associated with numerous systemic diseases, including cancer, type 2 diabetes (T2D), and heart failure (3). Reports since the 1960's have noted that elevations in circulating BCAAs tightly associate with insulin resistance (4).

The prevalence of diabetes in China has increased dramatically in the past two decades (5, 6). Elevated plasma branched chain amino acids (BCAAs) has been implicated in development of insulin resistance and T2D. However, whether consumption of BCAAs contribute to the disease is controversial. Some studies have shown that high intake of BCAAs is associated with an increased risk of T2D (2, 7, 8) and may have adverse effects on the development of IR (9). On the contrary, a study from a Japanese population reported that high intake of BCAAs may be associated with reduced diabetes risk (10). Research in this area has remained relatively limited. Thus, the association between dietary BCAAs and the risk of T2D in Chinese adults is unclear. Also, the quantity of BCAAs intake causing risk of T2D is not clearly defined. It could have significant clinical and public health implications that finding out exact BCAAs consumption threshold values of developing diabetes.

In the past few decades, dietary structure and food intakes of Chinese have undergone substantial changes (11). However, there is a lack of information on trends in BCAAs consumption and the risk of T2D associated with BCAAs intake, based on nationally representative data. Using data from 1997 to 2015 China Health and Nutrition Survey (CHNS) and China Nutrition and Health Survey (CNHS), the current study was aimed to systematically describe the changes in dietary BCAAs intake in Chinese adults from 1997 to 2015 and the risk of T2D caused by BCAAs intake.

METHODS

Study Population

All datasets used in this study were from two independent national project, CHNS and CNHS. CHNS was an international collaborative project cohosted by the Carolina Population Center at the University of North Carolina at Chapel Hill and the National Institute for Nutrition and Health (NINH) at Chinese Center for Disease Control and Prevention (CCDC), which aimed to examine the effects of the healthand nutrition. CNHS was a national survey conducted by the CCDC to survey the national health and nutrition status. The sampling method, dietary survey method, anthropometric measurement method, and quality control method of CNHS are almost identical to those of CHNS in terms of cross-section. The provincial staff for both projects are the same team. The core structure of the two surveys is the same in terms of cross-section. Both projects used stratified, multistage, random cluster sampling method, and further detailed information could be referred elsewhere (12, 13).

In the dietary BCAA transition trend analysis, seven cross-sectional data were obtained from CHNS (1997), CHNS (2000), CHNS (2004), CHNS (2006), CHNS (2009), CHNS (2011) and CNHS (2015). Data were included for analysis if dietary intake records were available and the age of the study object was 18 years or older at the time of survey. And data of 9,404, 10,291, 9,682, 9,553, 9,811, 12,686 and 71,695 participants in 1997, 2000, 2004, 2006, 2009, 2011, and 2015 were used for analysis, respectively.

In the BCAAs risk analysis, prospective cohort data were extracted from CHNS (1997–2015). Participants diagnosed with diabetes at baseline, those aged <18 years, and those without dietary records were excluded for analysis, and 15,508 participants with 9.9 ± 5.6 (mean ± SD) follow-up years were finally included for analysis.

Dietary BCAA Intake Assessment

BCAAs intake were calculated from 24-h dietary recall records and household condiment weighing records for three consecutive days (2 working days and 1 weekend). All field staff are professionally trained nutritionists who work in nutrition in their own county. BCAAs intakes was estimated by multiplying the consumed grams of each food by the amino acid contents of each food (referred from Chinese Food Composition Tables) (14–16) before BCAAs intake for all food items was summed by individual.

In the BCAA risk analysis, dietary exposure to BCAAs were calculated by using the average BCAAs intake values in each record before the onset of diabetes.

Identification of the New-Onset Diabetes

Since 1997, participants have been asked to report their previous diabetes history in the form of questionnaire interviews at each follow-up. Three questions were used to identify the new onset diabetes in the CHNS project: (1) Have the doctor told you that
you suffer from type 2 diabetes? (2) How old (age) were you when this happened? (3) Have you used the following treatment methods, such as special diet, weight control, oral medication, insulin injection, Chinese medicine, etc.? The diagnosis of T2D was based on patient-reported physicians’ diagnoses and/or the presence of diabetes-specific medication.

**Statistical Analysis**

We provided the demographic characteristics of each survey year. We also calculated the mean (SD) of dietary BCAAs by sex, age group and urban/rural status. A generalized linear model was used to test trends for consumption of BCAAs from 1997 to 2015, adjusting for sex, age, BMI and region. Heatmaps were generated and clustered using hierarchical clustering. For the comparison between the two groups, t-test was applied in Figure 1K, and generalized linear model was used in Table 4.

Based on the Cox proportional hazard model, a restricted cubic spline (RCS) curve was used to assess the association between dietary BCAAs levels and T2D risk on a continuous scale. In the statistical analyses, we adjusted for age, sex, energy intake, BMI, region, smoking status (previous or present, never), alcohol consumption (yes, no), which were well known risk factors for diabetes. In the Cox proportional hazards models, participants with previously diagnosed diabetes, were excluded when first entry into the survey. To balance best fit and overfitting in the main splines for incident diabetes, the number of knots, between three and six, was chosen as the lowest value for the Akaike information criterion, but if within two of each other for different knots, the lowest number of knots was chosen (17). In the non-linearity test, \( P < 0.1 \) was considered statistically significant for data exploration and visualization. Otherwise, two-sided significance tests were used throughout, and a two-sided \( P < 0.05 \) was considered statistically significant. All analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC) and R software, version 4.1.2.

**Patient and Public Involvement**

Participants were not involved in setting the research question or the outcome measures, nor were they involved in the design or implementation of the study. No participants were asked to advise on interpretation or writing of the manuscript.

**RESULTS**

From 1997 to 2015, the number of participants in the survey increased from 9,404 to 71,695, and the proportion of the elderly and urban residents continued to increase, reflecting increasing trends of aging and urbanization in China (Table 1). From 1997 to 2015, there was a significant decreasing trend in the BCAAs intake of Chinese adults in all subgroups (including the type of BCAAs, age subgroups, sex and urbanization status) \( (P < 0.0001) \) except for Leu in 80 or older, and a decreasing trend in the consumption of BCAAs after 40 years old \( (P < 0.05) \) (Table 2; Figure 1). From 1997 to 2015, cereals
continued to be the first primary source for dietary BCAA intake, but the proportion of its contribute decreased from 55.6% to 34.9%. Similarly, beans decreased from 10.1 to 7.2%. In contrast, the percent contribution of red meat increased from 9.5 to 17.5%. In addition, the contribution of fish and seafood increased from 6.5 to 8.6%, and eggs increased from 4.7 to 5.4%.

As shown in Figure 1J, the types of food were clustered into three major groups. Fish and seafood, red meat and poultry were clustered into one category. Cereals, eggs, vegetables, nuts, beans, and beverages were clustered into one category. Additionally, alcohol products, condiments, fruits, snacks, pickles and dairy products were clustered into one category. Dietary BCAAs (Leu, Ile, and Val) were clustered together with aspartate (Asp), histidine (His), glycine (Gly), arginine (Arg), threonine (Thr) and alanine (Ala). Furthermore, the top 4 types of food, exhibiting the strongest correlation with dietary BCAAs, were fish and seafoods, red meat, poultry and cereals.

At the endpoint of observation, mean BCAAs intake was higher in participants with new-onset diabetes onset than in non-diabetic participants ($t = -4.92, P < 0.0001$) (Figure 1K). The same phenomenon were also observed in Ile ($t = -4.62, P < 0.0001$), Leu ($t = -5.11, P < 0.0001$) and Val ($t = -4.78, P < 0.0001$).

The mean intake of BCAAs in the population of cohort study was 11.83 ± 3.77 g/day (Table 3). The impact of dietary BCAA intake on risk of T2D was shown in Figure 2. The consumption of BCAAs and risk of T2D was U-shape-associated and higher dietary BCAAs (≥14.01 g/day) increased the risk of T2D. When upon a closer look, higher intake of each BCAA also increased the risk of T2D (Figure 2). The 95% confidence interval (CI) was above the HR of 1.0, when the consumptions were higher than 14.01, 3.75, 6.07, 4.21 g/day in BCAAs, Ile, Leu and Val. Those with higher dietary BCAAs (Group B ≥ 14.01 vs. Group A < 14.01 g/day) also consumed more food in amounts (1616.96 ± 755.83 vs. 1244.92 ± 524.68 g/day, $P < 0.0001$) (Table 4). The average food intake of group A was 1244.92 (95% reference value 216.55 to 2273.29) g/day.

Compared with individuals with BCAAs consumption of 10.65–12.37 g/day, the multivariable-adjusted HR for diabetes was 2.26 (95% CI 1.45 to 3.51) for individuals with consumption of BCAAs more than 18.52 g/day (Table 5). The same trends were found in Ile and Leu, except for Val. The results were unaffected by multivariable adjustments in BCAAs, Ile and Leu.

### Sensitivity Analyses

When fractional polynomials was applied, the U-shaped association between dietary BCAAs intake and T2D risk also exist, and the BCAAs consumption cut-off that increased the T2D risk was 18.52 g/day (Table 5). When further stratified by sex and age, the association between the two was unaltered in men or in participants aged 45 years and older. However, the association between BCAAs intake and risk of T2D diminished in females or in participants younger than 45 years (Figures 2E–H).

### DISCUSSION

Using seven large-scale nationally representative survey data, a decreasing trend in dietary BCAAs intake was observed in the study population at all ages from 1997 to 2015. Consumption of BCAAs also declined as age increased for those aged 40 years older. In all food categories, the strongest correlations with BCAAs were with red meat, poultry, fish and seafoods. And the risk analysis showed that increased BCAAs intake was associated with an elevated risk of T2D. This association was more stable among men and among people with middle-age and elder. The people with risk of T2D accounted for about 23.86% of the total population due to BCAAs.

To the best of our knowledge, this is the largest study including the most recent national survey data to first address the dietary BCAAs intake trend and its risk on T2D. The reliability of our result could be guaranteed by the strict quality control of the CHNS and CNHS, including standardized protocols, standardized data collection procedures and standardized training of the field working stuff. This study contributes to the

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**TABLE 1 | Sociodemographic distribution of participants in the 1997–2015.**

| Age group (years) | 1997 | 2000 | 2004 | 2006 | 2009 | 2011 | 2015 |
|-------------------|------|------|------|------|------|------|------|
| Total             | 9,404 | 10,291 | 9,682 | 9,553 | 9,811 | 12,686 | 71,695 |
| Age group (years) |      |      |      |      |      |      |      |
| 18–34             | 3,339 (35.5) | 3,019 (29.3) | 2,142 (22.1) | 1,780 (18.6) | 1,692 (17.3) | 2,090 (16.5) | 8,695 (12.1) |
| 35–49             | 3,010 (32.0) | 3,636 (35.3) | 3,237 (33.4) | 3,156 (33.0) | 3,122 (31.8) | 3,967 (31.3) | 18,782 (26.2) |
| 50–64             | 1,949 (20.7) | 2,319 (22.5) | 2,782 (28.7) | 2,989 (31.3) | 3,208 (32.7) | 4,278 (33.7) | 27,697 (38.6) |
| 65–79             | 1,966 (10.2) | 1,157 (11.2) | 1,333 (13.8) | 1,412 (14.8) | 1,518 (15.5) | 2,004 (15.8) | 14,793 (20.6) |
| 80 or older       | 150 (1.6) | 160 (1.6) | 188 (1.9) | 216 (2.3) | 271 (2.8) | 347 (2.7) | 1,782 (2.5) |
| Sex (%)           |      |      |      |      |      |      |      |
| Male              | 4,562 (48.5) | 4,980 (48.4) | 4,614 (47.7) | 4,538 (47.5) | 4,676 (47.7) | 5,933 (46.8) | 34,140 (47.6) |
| Female            | 4,842 (51.5) | 5,311 (51.6) | 5,068 (52.3) | 5,015 (52.5) | 5,135 (52.3) | 6,753 (53.2) | 37,555 (52.4) |
| Living area (%)   |      |      |      |      |      |      |      |
| Urban             | 2,971 (31.6) | 3,256 (31.6) | 3,007 (31.1) | 2,984 (31.2) | 3,082 (31.4) | 5,281 (41.6) | 29,145 (40.7) |
| Rural             | 6,433 (68.4) | 7,035 (68.4) | 6,675 (68.9) | 6,669 (68.8) | 6,729 (68.6) | 7,405 (58.4) | 42,550 (59.4) |

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Yu et al. Dietary BCAAs Transition and Diabetes
Table 2: Trends in mean BCAAs intake among Chinese adults from 1997 to 2015.

| Year | Ile (mg/day) | Leu (mg/day) | Val (mg/day) | BCAAs (mg/day) |
|------|-------------|-------------|-------------|----------------|
| 1997 | 3165.81 (1124.99) | 5449.48 (1995.36) | 3657.37 (1282.00) | 12272.66 (4363.69) |
| 2000 | 3102.89 (1122.43) | 5277.97 (1947.35) | 3520.52 (1242.34) | 12272.66 (4363.69) |
| 2004 | 3004.50 (1189.17) | 5140.51 (2076.71) | 3456.76 (1344.52) | 11901.38 (4280.58) |
| 2009 | 3018.33 (1172.68) | 5155.99 (2036.53) | 3427.45 (1305.43) | 11601.77 (4737.22) |
| 2011 | 3063.48 (1181.62) | 5261.45 (2056.60) | 3489.38 (1313.68) | 11814.32 (4532.88) |
| 2015 | 2805.68 (1159.1) | 4995.45 (2110.14) | 3248.66 (1314.82) | 11049.79 (4554.87) |

P for trend: <0.0001

Δ: 530.37

SD, standard deviation. Linear trends in the mean BCAAs intake from 1997 to 2015 were tested using generalized linear model adjusted for sex, age, BMI and region. Δ 1997–2015.
The declines in BCAs intake may well have contributed to the declining T2D morbidity. According to a recent study, the incidence of diabetes decreased from 2007 to 2017 in both men and women in China (18). And, the trend in consumption of BCAs paralleled with the decreased trend of T2D incidence, which reduced by 14.36% from 1997 to 2015 in the adult population (Table 2). The declined BCAs intake reflected changes of society and behavioral lifestyle in China. Accompanying with the decreasing BCAs consumption, it was also observed that energy and protein intake decreased substantially from 1992 to 2012 among Chinese adults (11). One possible reason for these declines could be decreased physical activity. In China, although leisure-time physical activity have generally increased since 2000 (19), total physical activity have dropped sharply from 1991 to 2009 (20), and classical literatures showed a J-shaped relationship between physical activity and energy intake (21, 22). However, physical activity was also inversely related to incident diabetes (23). Still, the age-standardized incidence rates of diabetes subsequently decreased from 2007 to 2017 (18).

In dietary BCAs risk analysis of the cohort, increased dietary BCAs intake was associated with an elevated risk of T2D. Men and older people were more sensitive to the risk of diabetes caused by BCAs. The conclusions reached in this study were similar to previous studies in the US and northeastern China (2, 7, 8). In the prospective cohort study of United States, HR of diabetes for the highest quintile of BCAs intake compared with the lowest quintile were 1.13 (95%CI, 1.07–1.19, P < 0.001) in leucine, 1.13 (95%CI, 1.07–1.19, P < 0.001) in isoleucine and 1.11 (95%CI, 1.05–1.17, P < 0.001) in valine (2). In Harbin, China and the American population, it has been observed that higher dietary BCAs intake will promote the risk of T2D. The Harbin population study showed that the OR and 95% CI across quartiles of total BCAs intakes for T2D within the 4th quartile were 1.0, 1.337 (0.940–1.903); 1.579 (1.065–2.343); 2.412 (1.474–3.947) (8). In a meta-analysis study, higher total intake of BCAs causes increased T2DM risk with an OR and 95% CI of 1.32 (1.14, 1.53) (24). However, the results may seem in contrast to the study from Japan (10). The Japanese study showed that increased intake of BCAs may be associated with a reduced risk of diabetes. The HR between the highest tertile and the lowest tertile was 0.70 (95% CI: 0.48–1.20, P for trend = 0.06). In that study, total BCAs, leucine and valine intakes were inversely associated with T2D risk in women, and no associations were found in men. Studies have shown that dietary BCAs affect human metabolism and the risk of chronic diseases (25). A study of young people in northern China showed that a higher dietary BCAs ratio was negatively correlated with postprandial blood glucose (26). Reducing the intake of dietary BCAs can improve glucose tolerance and body composition (27, 28). Although studies have shown that dietary BCAs were closely related to multiple chronic diseases, this paper bridges a gap in large cohort studies of representative populations of Chinese.

Of serum BCAs levels, 80% were determined by protein or BCAs from diet or supplements, and the remaining 20% are related to their catabolites (29, 30). Studies have shown that oral BCAs supplementation can affect the leucine content in blood circulation. The relationship between serum BCAs levels and the occurrence and development of chronic diseases were well established. Studies have found that elevated levels of serum BCAs are closely related to weight gain, insulin resistance, and abnormal glucose metabolism in adults (31, 32). Animal experiments have shown that in non-obesity, insulin resistance, and fructose-fed rat models, elevated serum BCAs levels were associated with insulin resistance (33). Previous studies also showed higher plasma levels of BCAs were associated with

### TABLE 3 | Baseline characteristics of 15,508 individuals in the CHNS Study.

| No. of individuals | Age | Weight | Height | Drinker | Smoker | Systolic blood pressure (mm Hg) | Diastolic blood pressure (mm Hg) | Triceps skin fold (mm) | Hip Circumference (cm) | Waist Circumference (cm) | BCAAs intake (g/day) | Analysis | 1st-5th | 6th-20th | 21st-40th | 41st-60th | 61st-80th | 81st-95th | 96th-100th | All |
|-------------------|-----|--------|--------|---------|--------|-------------------------------|-------------------------------|----------------------|----------------------|------------------------|----------------------|----------|---------|---------|---------|---------|---------|---------|---------|
| 778 (5.0)         | 50.6 (17.3) | 57.3 (11.5) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |
| 547 (70.3)        | 50.6 (17.3) | 57.3 (11.1) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |
| 50.6 (17.3)       | 50.6 (17.3) | 57.3 (11.1) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |
| 50.6 (17.3)       | 50.6 (17.3) | 57.3 (11.1) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |
| 50.6 (17.3)       | 50.6 (17.3) | 57.3 (11.1) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |
| 50.6 (17.3)       | 50.6 (17.3) | 57.3 (11.1) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |
| 50.6 (17.3)       | 50.6 (17.3) | 57.3 (11.1) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |
| 50.6 (17.3)       | 50.6 (17.3) | 57.3 (11.1) | 157.5 (8.3) | 23 (3.9) | 174 (22.4) | 152.4 (20.3) | 79.1 (11.6) | 16.5 (8.1) | 80.6 (10.6) | 26.3 (4.5) | 5.14 (1.18) | 113.0 (95%CI, 1.07–1.19, P < 0.001) |

Values are means (standard deviation) or number (%). BCAs, Branched-Chain Amino Acids.
FIGURE 2 | Multivariable adjusted hazard ratios of incident type 2 diabetes according to levels of BCAAs consumption on a continuous scale in the overall population. Solid blue lines are multivariable adjusted hazard ratios, with dashed blue lines showing 95% confidence intervals derived from restricted cubic spline regressions with three knots. Reference lines for no association are indicated by solid bold lines at a hazard ratio of 1.0. Dashed yellow curves show fraction of population with different levels of BCAAs intake. Arrows indicate the lowest consumption of BCAAs and fraction of population with risk of T2D. Analyses were adjusted for age, sex, (Continued)
FIGURE 2 | smoking status, alcohol consumption, BMI, physical activity levels and energy intake at baseline. Based on individuals from the CHNS followed for a mean 9.9 years. **(A–D)** Representation of restricted cubic spline cox regression models for dietary BCAAs, Ile, Leu, Val and risk of type 2 diabetes. **(E–H)** Representation of restricted cubic spline cox regression models for dietary BCAAs and risk of type 2 diabetes in different age and gender subgroups.

TABLE 4 | Differences in diet with upper and lower thresholds of BCAAs.

| Variables                  | Group A BCAAs < 14.01 g/day (n = 11,808) | Group B BCAAs ≥ 14.01 g/day (n = 3,700) | \( \chi^2/F \) | P-value |
|----------------------------|------------------------------------------|------------------------------------------|----------------|---------|
| **n (%)/Mean (SD)**        |                                          |                                          |                |         |
| **Demographic characteristics** |                                          |                                          |                |         |
| Female                     | 6,800 (57.59%)                           | 1,357 (36.88%)                           | 494.1568       | <0.0001 |
| Age                        | 44.43 (15.16)                            | 41.07 (13.48)                            | 127.0792       | <0.0001 |
| ≥60 years                  | 2,161 (18.30%)                           | 386 (10.43%)                             | –6.03          | <0.0001 |
| BMI                        | 22.85 (3.37)                             | 23.25 (3.45)                             |                |         |
| Smoking history            | 3,371 (28.55%)                           | 1,493 (40.35%)                           | 182.3113       | <0.0001 |
| Alcohol consumption history| 3,685 (31.21%)                           | 1,735 (46.89%)                           | 304.8290       | <0.0001 |
| Energy intake              | 2,042.12 (707.20)                        | 2,593.34 (1,215.11)                     | –26.23         | <0.0001 |
| **Food categories (g/day)** |                                          |                                          |                |         |
| Cereals                    | 451.00 (194.16)                          | 526.54 (262.78)                          | 206.97         | <0.0001 |
| Beans                      | 53.51 (64.61)                            | 84.83 (90.13)                            | 329.59         | <0.0001 |
| Vegetables                 | 393.83 (166.80)                          | 453.22 (253.42)                          | 166.45         | <0.0001 |
| Pickles                    | 3.39 (10.05)                             | 3.77 (10.34)                             | 7.47           | 0.0063  |
| Fruits                     | 59.74 (104.03)                           | 75.91 (126.55)                           | 27.23          | <0.0001 |
| Nuts                       | 4.24 (14.06)                             | 8.69 (23.29)                             | 149.07         | <0.0001 |
| Red meat                   | 74.94 (68.94)                            | 121.36 (116.93)                          | 568.14         | <0.0001 |
| Poultry                    | 13.75 (29.13)                            | 30.87 (52.40)                            | 445.93         | <0.0001 |
| Dairy products             | 17.19 (58.41)                            | 37.23 (91.20)                            | 169.43         | <0.0001 |
| Eggs                       | 31.09 (34.04)                            | 45.86 (49.85)                            | 247.45         | <0.0001 |
| Fish and seafoods          | 29.49 (45.91)                            | 66.49 (81.14)                            | 982.46         | <0.0001 |
| Snacks                     | 11.24 (37.46)                            | 15.43 (51.95)                            | 8.52           | 0.0035  |
| Sugar and starch           | 2.91 (10.40)                             | 3.87 (13.70)                             | 6.75           | 0.0094  |
| Sauce                      | 0.52 (3.12)                              | 0.43 (2.65)                              | 2.27           | 0.1321  |
| Alcohol products           | 10.93 (55.62)                            | 27.27 (104.20)                           | 58.74          | <0.0001 |
| Fast food                  | 10.32 (37.51)                            | 17.08 (62.32)                            | 17.47          | <0.0001 |
| Beverage                   | 3.26 (60.59)                             | 9.84 (58.11)                             | 21.2           | <0.0001 |
| Vegetable oil and condiments| 56.17 (32.71)                           | 64.25 (35.98)                            | 72.53          | <0.0001 |
| Others                     | 17.43 (38.21)                            | 24.21 (64.06)                            | 26.63          | 0.0004  |
| Total food intake          | 1244.92 (524.68)                         | 1616.96 (755.83)                         | 671.59         | <0.0001 |

SD, standard deviation. Demographic characteristics were tested using Chi-square test and student’s t-test. Group differences of food consumption were calculated using the generalized linear models after adjusting for age, sex, energy intake, BMI, region, physical activity levels, smoking status (previous or present, never), alcohol consumption (yes, no).

an increased risk of T2D (34, 35). Prospective population studies have proved that serum BCAA levels can predict the future risk of diabetes (36). In patients with overweight and metabolic syndrome, there was also a correlation between plasma BCAA levels and red meat or animal protein (37). Therefore, control of serum BCAAs can start from dietary BCAAs intake. Our results link dietary BCAAs with population health, especially the risk of diabetes. In this study, group B (BCAAs ≥ 14.01 g/day) was significantly higher than group A (BCAAs < 14.01 g/day) in total food intake and most food categories (\( P < 0.0001 \), Table 4). From this point of view, high consumption of BCAAs is accompanied by high consumption of food. Our results are in accordance with a recent study. When the quantity of food intake exceeded certain thresholds, the risks of new-onset diabetes increased or reached a plateau (38).

In all food categories, the strongest correlations with BCAAs were with red meat, poultry, fish and seafoods. Our research found that although BCAA intake is decreasing, sources have changed over time. Now animal sources are main sources and previously cereals. Meanwhile, there was also a correlation between plasma BCAA levels and red meat or animal protein (37). A similar phenomenon was also found in the Brazilian population that the main food sources of BCAA were unprocessed red meat, unprocessed poultry, bread and toast, beans and rice (39). Epidemiological studies have shown that high consumption of animal protein, especially red meat with high levels of methionine and BCAAs, have promoted the progression...
TABLE 5 | Hazard ratios for incident type 2 diabetes according to categories of levels of BCAAs (Ile, Leu, Val) intake, sex and age adjusted, and multivariable adjusted.

| Centile | Consumption (g/day) | Individuals | Events | Event rate per 1,000 person years | Age and sex adjusted hazard ratio (95% CI) | Hazard ratio (95% CI) | Multivariable adjusted hazard ratio (95% CI) | Hazard ratio (95% CI) |
|---------|---------------------|-------------|--------|------------------------------------|-------------------------------------------|----------------------|---------------------------------------------|----------------------|
| BCAAs   |                     |             |        |                                    |                                           |                      |                                             |                      |
| 1st-5th | <6.44               | 778         | 21     | 4.98                               |                                           | 1.25 (0.77–2.03)     | 1.72 (1.03–2.88)                             |                      |
| 6th-20th| 6.44–8.82           | 2,330       | 61     | 2.97                               |                                           | 0.86 (0.62–1.20)     | 1.12 (0.78–1.6)                              |                      |
| 21st-40th| 8.82–10.65          | 3,091       | 108    | 3.32                               |                                           | 1.20 (0.91–1.59)     | 1.43 (1.05–1.95)                             |                      |
| 41st-60th| 10.65–12.37         | 3,113       | 90     | 2.54                               |                                           | 1.0                  | 1.0                                         |                      |
| 61st-80th| 12.37–14.53         | 3,096       | 106    | 3.05                               |                                           | 1.27 (0.96–1.68)     | 1.35 (0.99–1.84)                             |                      |
| 81st-95th| 14.53–18.52         | 2,324       | 79     | 3.38                               |                                           | 1.47 (1.08–1.99)     | 1.27 (0.9–1.78)                              |                      |
| 96th-100th| >18.52              | 776         | 31     | 5.56                               |                                           | 2.46 (1.63–3.72)     | 2.26 (1.45–3.51)                             |                      |
| Ile     |                     |             |        |                                    |                                           |                      |                                             |                      |
| 1st-5th | <1.64               | 769         | 22     | 5.41                               |                                           | 1.43 (0.88–2.30)     | 1.84 (1.11–3.05)                             |                      |
| 6th-20th| 1.64–2.27           | 2,332       | 68     | 3.38                               |                                           | 1.03 (0.74–1.42)     | 1.27 (0.89–1.79)                             |                      |
| 21st-40th| 2.27–2.75           | 3,069       | 103    | 3.20                               |                                           | 1.16 (0.88–1.55)     | 1.29 (0.94–1.76)                             |                      |
| 41st-60th| 2.75–3.20           | 3,124       | 89     | 2.50                               |                                           | 1.0                  | 1.0                                         |                      |
| 61st-80th| 3.20–3.77           | 3,131       | 106    | 3.01                               |                                           | 1.27 (0.96–1.68)     | 1.28 (0.94–1.75)                             |                      |
| 81st-95th| 3.77–4.77           | 2,303       | 77     | 3.26                               |                                           | 1.45 (1.06–1.97)     | 1.24 (0.88–1.74)                             |                      |
| 96th-100th| >4.77               | 780         | 31     | 5.43                               |                                           | 2.44 (1.62–3.69)     | 2.14 (1.38–3.32)                             |                      |
| Leu     |                     |             |        |                                    |                                           |                      |                                             |                      |
| 1st-5th | <2.83               | 783         | 19     | 4.34                               |                                           | 1.07 (0.65–1.78)     | 1.37 (0.80–2.35)                             |                      |
| 6th-20th| 2.83–3.88           | 2,305       | 62     | 3.02                               |                                           | 0.88 (0.63–1.23)     | 1.28 (0.90–1.84)                             |                      |
| 21st-40th| 3.88–4.72           | 3,126       | 107    | 3.22                               |                                           | 1.18 (0.89–1.57)     | 1.52 (1.11–2.07)                             |                      |
| 41st-60th| 4.72–5.51           | 3,110       | 89     | 2.51                               |                                           | 1.0                  | 1.0                                         |                      |
| 61st-80th| 5.51–6.49           | 3,081       | 107    | 3.13                               |                                           | 1.32 (1.00–1.76)     | 1.46 (1.07–2.00)                             |                      |
| 81st-95th| 6.49–8.34           | 2,330       | 78     | 3.37                               |                                           | 1.46 (1.07–1.98)     | 1.32 (0.93–1.86)                             |                      |
| 96th-100th| >8.34               | 773         | 34     | 6.16                               |                                           | 2.72 (1.83–4.06)     | 2.67 (1.74–4.11)                             |                      |
| Val     |                     |             |        |                                    |                                           |                      |                                             |                      |
| 1st-5th | <1.93               | 785         | 21     | 4.96                               |                                           | 1.20 (0.74–1.95)     | 1.60 (0.96–2.66)                             |                      |
| 6th-20th| 1.93–2.62           | 2,323       | 67     | 3.33                               |                                           | 0.93 (0.67–1.28)     | 1.19 (0.84–1.68)                             |                      |
| 21st-40th| 2.62–3.16           | 3,117       | 101    | 3.08                               |                                           | 1.05 (0.79–1.39)     | 1.21 (0.89–1.65)                             |                      |
| 41st-60th| 3.16–3.66           | 3,062       | 93     | 2.67                               |                                           | 1.0                  | 1.0                                         |                      |
| 61st-80th| 3.66–4.30           | 3,131       | 108    | 3.06                               |                                           | 1.21 (0.92–1.60)     | 1.25 (0.92–1.70)                             |                      |
| 81st-95th| 4.30–5.44           | 2,314       | 77     | 3.28                               |                                           | 1.37 (1.01–1.86)     | 1.32 (0.95–1.85)                             |                      |
| 96th-100th| >5.44               | 776         | 29     | 5.13                               |                                           | 2.17 (1.43–3.31)     | 1.45 (0.90–2.33)                             |                      |

Multivariable adjusted analyses were adjusted for age, sex, smoking status, alcohol consumption, BMI, physical activity levels and energy intake at baseline. Based on individuals from the CHNS followed for a mean 9.9 years. Interaction with consumption of red meat (P-value for interaction > 0.05), fish and sea foods (P-value for interaction > 0.05), poultry (P-value for interaction > 0.05).
of age-related diseases (40). And, reducing BCAAs consumption in the Western diet improved glucose tolerance and relieved insulin resistance. Previous research has indicated that reducing dietary BCAAs may represent a highly translatable option for the treatment of obesity and insulin resistance in animals (41). According to the results of this study, we propose dietary recommendations for the population’s diet to prevent diabetes. The dietary intake should not exceed 2,273 g/day, and the intake of red meat, poultry, fish and seafoods should be controlled at the same time.

Our study also has several limitations. First, these surveys are not carried out annually, which could have allowed more details in trends. Second, dietary consumption data from the CHNS survey 2015 was not available. We used the dietary information from CNHS survey 2015 for make-up. Statistical processing was used to ensure the quality of the results and the comparability between the CNHS and CHNS. Third, our dietary intake estimates are mainly based on 3-day 24-h meal recall, so measurement errors are inevitable. In order to reduce selection biases and measurement errors, we averaged three 24-h dietary recalls for different age groups or urban/rural areas. The average long-term intakes were used to represent the dietary exposure level of the participants. Finally, when the CHNS survey was planned and implemented, the State Statistical Office of China would not share their sample frame with the CHNS team. Furthermore, the data sets for public distribution would not be released if the CHNS team had worked with them. However, the design used extant census data as best as we could for a multi-level random sample.

In conclusion, a trend toward decreased BCAAs intake was observed in Chinese of all subgroups (including age and sex) from 1997 to 2015. After 40 years of age, consumption of BCAAs declined with increasing age. In all food categories, the strongest correlations with BCAAs were with red meat, poultry, fish and seafoods. Higher BCAAs intake was associated with higher risk of T2D. This relationship is more stable among men and middle-aged and elderly people. The people with risk of T2D accounted for about 23.86% of the total population due to BCAAs. Based on the results of this study, in order to prevent diabetes, we recommend that dietary intake should be restricted, while controlling the intake of red meat, poultry, fish and seafood.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the copyright of the dataset is currently owned by the Chinese Center for Disease Control and Prevention and has not been fully disclosed yet. Requests to access the datasets should be directed to https://www.cpc.unc.edu/projects/china.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JZ is guarantor, designed the study, principal investigator, and attests that all the listed authors meet the authorship criteria and that no others meeting the criteria have been omitted. LY conducted the data analysis and drafted the manuscript. PS, QZ, YL, SJ, SZ, and ZW critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the final version of the manuscript.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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