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Dietary intake of branched-chain amino acids and pancreatic cancer risk in a case-control study from Italy

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Short title: Dietary BCAAs and pancreatic cancer

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Abstract

Circulating branched-chain amino acids (BCAAs), a subgroup of the nine essential amino acids, have been associated to pancreatic cancer risk. The aim of this study is to estimate the relation between BCAA intake from diet and pancreatic cancer risk.

We analysed data from a multicentric Italian case-control study, including 326 pancreatic cancer cases and 652 controls, matched to cases by study centre, sex and age. A validated food-frequency questionnaire was used to collect the participants' usual diet before cancer diagnosis (or hospital admission for controls) and to compute dietary intakes of various nutrients, including BCAAs. Odds ratios (ORs) and corresponding confidence intervals (CIs) were computed through logistic regression models conditioned on the matching variables and adjusted for major confounding factors, including total energy intake.

We found a positive association between the BCAA intake and pancreatic cancer risk (OR for the third quartile=1.88, 95% CI=1.08-3.26; OR for the fourth quartile =2.17, 95% CI=1.17-4.06), with a significant trend in risk. The association persisted after excluding subjects with diabetes and family history of pancreatic cancer, and across strata of selected covariates.

These data support and quantify the association between dietary BCAAs and pancreatic cancer, previously suggested by studies on circulating BCAAs.

Keywords: Pancreatic cancer; Branched-chain amino acids; Diet; Case-control study; Risk factors.

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INTRODUCTION

Pancreatic cancer is a common cancer with poor prognosis, ranking as the fourth cause of cancer death in both sexes combined in the EU, and it is one of the few cancers for which mortality has not declined over the last three decades [1; 2].

Tobacco smoking and diabetes are the best recognized risk factors for pancreatic cancer [3; 4; 5]. High alcohol intake was also associated to excess pancreatic cancer risk [6; 7]. Some of the risk factors for this malignancy, such as obesity, waist circumference, and diabetes, are strongly related to diet [8; 9; 10; 11; 12; 13]. A western dietary pattern, high in animal products and red meat, has been shown to increase pancreatic cancer risk [14; 15; 16].

The branched-chain amino acids (BCAAs), i.e. leucine, isoleucine and valine, are a subgroup of the nine essential amino acids. They derive from protein food sources, mainly animal food products such as meat, fish, and dairy products [10; 17].

BCAAs have been associated to various cardiometabolic conditions such as type 2 diabetes, insulin resistance, and adiposity [17; 18; 19; 20; 21; 22]. They have also been associated with the risk of selected cancers, including those of the colorectum and the breast [23; 24; 25]. Three epidemiological studies investigated the relationship between BCAAs and pancreatic cancer risk, reporting positive associations with circulating BCAA plasma levels, but no data were available on BCAA estimates from diet to date [24; 26; 27]. In addition, in vivo and in vitro studies support the implication of BCAAs in the development and progression of pancreatic cancer [28; 29; 30].

We analyzed the relationship between dietary BCAA intake and pancreatic cancer risk in an Italian multicentric study.

METHODS

We used data from a multicentric case-control study of pancreatic cancer, conducted between 1991 and 2008 in the province of Pordenone and in the greater Milan area, northern Italy [15]. The study included 326 cases (174 men, 152 women, median age 63 years, range 34–80 years) with incident cancer of the pancreas. Eighty-four per cent of cases were interviewed within one month from diagnosis and the remaining cases within one year. Controls were 652 patients (348 men, 304 women, median age 63 years, range 34–80 years) admitted to the same teaching or general hospitals as cases for a wide spectrum of acute nonneoplastic conditions, unrelated with digestive tract diseases, smoking, alcohol consumption, or long-term modifications of diet. Controls were hospitalized for traumatic orthopedic disorders

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(31%), other orthopedic disorders (31%), acute surgical conditions (28%), and miscellaneous other illnesses (10%), including eye, nose, ear, skin, or dental disorders (10%). Controls were frequency-matched to cases by study centre, sex and age ($\pm$ 5 years), with a control to case ratio of 2:1. Less than 5% of the approached cases and controls refused to participate in the study. All enrolled subjects signed an informed consent, according to the recommendations of the Board of Ethics of each participating centre. All procedures were performed in accordance with the ethical standards according to the Declaration of Helsinki.

Cases and controls were interviewed in hospital by centrally trained interviewers, with the use of a standard, structured questionnaire. The questionnaire included information on sociodemographic and anthropometric factors at different ages, selected lifestyle habits, such as history of tobacco use, and physical activity, personal medical history of selected diseases including diabetes, and family history of cancer in first degree-relatives.

For the dietary assessment during the years preceeding cancer diagnosis or hospital admission, a validated and reproducible food frequency questionnaire (FFQ) was used [31; 32]. Subjects were asked to indicate their average weekly consumption of 78 food items, food groups or recipes. Data on history of consumption of alcoholic beverages were also collected in an additional section that includes 5 items (resulting in a total of 83 FFQ items).

We used an Italian food composition database to estimate total energy, nutrients, calcium, vitamin D and BCAA intake of study participants [33; 34]. Data on BCAAs were available for leucine, isoleucine and valine. Given the high collinearity between leucine, isoleucine and valine (r~1.00), we analyzed total BCAA intake as the sum of their individual intakes.

BCAA intake was categorized into quartiles based on the distribution of controls, both directly on the BCAA intakes and on the residuals of the regression of BCAAs on energy [35]. Since both analyses yielded similar results, only findings from the first approach are presented. Using the lowest quartile as reference, odds ratios (ORs) of pancreatic cancer for BCAA quartiles and the corresponding 95% confidence intervals (CIs) were estimated by logistic regression models, conditioned on study centre, sex and age, and further adjusted for year of interview (continuous variable), years of education (<7, 7-11, $\geq$12; categorically), body mass index (BMI, <22, 22-24.9, 25-29.9, $\geq$30 kg/m^2; categorically), cigarette smoking (never smoker, former smoker, current smoker of <15 and $\geq$15 cigarettes per day; categorically), history of diabetes (yes, no), family history of pancreatic cancer (yes, no), alcohol intake (never drinker, ever drinker of <7, 7– 20.9, and $\geq$21 drinks per week; categorically) and total energy intake (tertiles; categorically). We also run unconditional regression models and found no appreciably changes in the OR estimates. Tests for trend

were based on the likelihood ratio test between models with and without a linear term for BCAAs. We further adjusted the OR by including in turn in the model terms for other dietary factors both correlated with BCAA intakes (r>0.50) and previously associated to pancreatic cancer risk [15; 34; 36], including vegetable protein (r~0.69), total lipids (r~0.77), fibre (r~0.51), folate (r~0.69), vitamin E (r~0.56), vitamin D (r~0.58), and calcium (r~0.74) intakes, and red meat (r~0.64).

Sensitivity analyses were performed excluding in turn subjects with diabetes, with family history of pancreatic cancer, and with outliers in energy intake (<500 or $\geq$ 4000 kcal/day).

We computed the ORs for BCAA quartiles in strata of sex, age (< 60 and $\geq$ 60 years), BMI (<25 and $\geq$25 m/kg2), smoking status (non and current smokers), and alcohol consumption (no or light: <7, moderate: 7-20.9, heavy: $\geq$ 21 drinks/week). We estimated heterogeneity between strata through the likelihood ratio test comparing the models with and without interaction terms.

RESULTS

Table 1 shows the distribution of 326 pancreatic cancer cases and 652 controls according to sex, age and other characteristics. By design, cases and controls had similar distributions for sex, age and centre. Cases were more frequently interviewed after 2000 than controls but there was no cluster of cases and/or controls in any specific calendar year. Cases were more educated, more frequently smokers and reported more frequently a history of diabetes than controls.

Table 2 gives the number and percentage of cases and controls, the ORs and their corresponding 95% CI according to quartiles of BCAA intake, with the lowest quartile as reference category. We found a positive association between the BCAA intake and pancreatic cancer risk from the third quartile onward (OR for the third quartile=1.88, 95% CI=1.08-3.26; OR for the fourth quartile =2.17, 95% CI=1.17-4.06), with a significant trend in risk.

Table 3 shows the ORs for BCAA intake quartile, compared to the lowest one, after adjustment in turn for selected dietary factors. Allowance for vegetable protein, lipids, fibre, folate, vitamin E, vitamin D, and calcium intake, as well as for red meat consumption, did not appreciably modify the association.

After exclusion of 47 cases and 37 controls with a history of diabetes, the ORs for the highest quartile of BCAA intake compared with the lowest one slightly increased to 2.49 (95% CI=1.29-4.83). Similarly, after exclusion of 10 cases and 15 controls with family history of pancreatic cancer, the OR became 2.41 (95% CI=1.28-4.55). When we excluded 17 cases and

19 controls with outliers in energy intake, the OR became 2.06 (95% CI= 1.09-3.88) (data not shown).

Table 4 shows the relation between BCAA intake and pancreatic cancer risk in strata of sex, age, BMI, smoking status, and alcohol consumption. The associations were apparently stronger in men, young individuals, those with higher BMI, in smokers and higher alcohol intake, although in the absence of significant heterogeneity.

We also evaluated the combined effect of dietary BCAA exposure and lifestyle behaviours, such as tobacco status and alcohol consumption on pancreatic cancer risk (Figure 1). Using as reference group non-smokers in the lowest two quartiles of BCAA intake (102 cases and 259 controls), the ORs in the two highest quartiles of BCAA intake were 1.44 (95% CI=0.91-2.30) among non-smokers (121 cases and 258 controls), and 3.64 (95% CI=1.97-6.73) among current smokers of ≥15 cigarettes/day (47 cases and 40 controls) (Figure 1 A). Using as reference group never/light alcohol drinkers (0⊦ - <7 drinks/week) in the first two quartiles of BCAA intake (48 cases and 133 controls), never/light alcohol drinkers in the two highest BCAA quartiles (39 cases and 86 controls) had an OR of 1.68 (95% CI=0.88-3.20) and heavy alcohol drinkers (≥ 21 drinks/week) in the two highest BCAA quartiles (90 cases and 119 controls) had an OR of 4.57 (95% CI=2.28-9.15) (Figure 1 B).

DISCUSSION

Our study found a positive association between total dietary BCAA intake and pancreatic cancer risk, after adjusting for several potential confounding factors, including tobacco smoking, BMI and history of diabetes, and additional nutritional factors. The excess risk became substantial in combination with high tobacco or alcohol consumption.

To our knowledge, no previous study evaluated the relation between dietary BCAA intake and pancreatic cancer risk. Higher circulating BCAA levels have been positively associated with pancreatic cancer risk in different study populations. In a prospective study on four large U.S. cohorts, including 454 pancreatic cancer cases, elevated plasma levels of BCAAs was associated with a 2–fold increased risk of pancreatic cancer. The OR in the highest quintile of total plasma BCAAs compared with the lowest one was 2.01 (95% CI, 1.34-3.03) [27]. When authors considered separately BCAA plasma levels, they found similar ORs for leucine (OR=1.97; 95% CI=1.29-2.99), isoleucine (OR= 2.00; 95% CI=1.31-3.05), and valine (OR=1.90, 95% CI=1.28-2.81). Interestingly, the strongest associations between BCAA levels and pancreatic cancer risk were found in subjects with blood samples collected 2 to 5

years before diagnosis (OR for total BCAAs= 4.34; 95% CI=1.82–10.35) compared to more than 5 years [27]. In a nested case-control study within the Japan Public Health Center-based prospective study, including 170 cases of pancreatic cancer, the OR for the highest serum BCAA quartile, compared with the lowest one, was 2.43 (95% CI=1.21–4.90) [26]. The ORs were 2.14 (95% CI =1.10-4.15) for leucine and 2.89 (95% CI=1.43-5.84) for valine. In the longitudinal Women's Health Study (WHS) cohort of 26,711 US women including 74 cases of pancreatic cancer, circulating total BCAAs were marginally associated with pancreatic cancer risk (Hazard Ratio, HR, per one standard deviation=1.24; 95% CI=0.98-1.57) [24]. The HRs were 1.27 (95% CI=0.99-1.64) for leucine, 1.31 (95% CI=1.01,1.07) for isoleucine, and 1.13 (95% CI=0.89-1.43) for valine [24]. We were not able to investigate individual BCAAs, given the high collinearity of their intakes in our data. Comparing findings from circulating BCAA levels and dietary intakes needs caution considering the low agreement found between these measures [37]. However, our results are in line with previous studies on circulating BCAAs providing further evidence for a positive association between leucine, isoleucine and valine and pancreatic cancer risk.

Different mechanisms have been implicated for this association. In a murine model of pancreatic cancer, leucine supplementation reduced glucose clearance in obese mice with subsequent increase in circulating glucose enhancing pancreatic tumor growth [29]. Moreover, pancreatic tumor tissues showed increased BCAA uptake through solute carrier transporters [28]. Knockdown of the key enzymes in BCAA catabolism, BCAT2 and BCKDHA, inhibited pancreatic ductal adenocarcinoma cell proliferation, by regulating lipogenesis which has been shown to be increased in proliferative cells. These need high levels of fatty acids for the generation of the cell membranes. In an animal study, KRAS mutation, a signature marker occurring in more than 90% adenocarcinoma of pancreas, was found to be positively correlated with BCAT2 protein level [30]. In the same study, pancreatic cancer cells consumed 1.5 to 2.5 times more BCAAs than normal cells [30].

Major food sources of BCAA intake in our data were red meat (26%), dairy products (13%), poultry (12%) and fish (7%). In this study, red meat and cheese were positively associated to pancreatic cancer risk (ORs for high versus low consumption=1.99, 95% CI= 1.18-3.36 and OR=1.90, 95% CI= 1.12-3.19, respectively), whereas no association was found for poultry (OR= 0.91; 95% CI= 0.70-1.58) and fish (OR=1.05; 95% CI= 0.71-1.56) [15; 16]. Several studies have shown positive associations between red meat and pancreatic cancer risk [36; 38]. Also, dietary patterns with low intake of animal protein such as the Mediterranean diet [39] or a posteriori-derived healthy dietary pattern (non-western type diet) [14; 40] have been shown to

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exert a protective effect on the risk of pancreatic cancer. Since in our population BCAA intake mainly derived from animal food sources with a small contribution from fish consumption, it would be of interest to evaluate the relation between BCAA intake and pancreatic cancer risk in populations with a high consumption of fish, such as Nordic countries or Japan. However, in this study, further allowance for dietary factors correlated with BCAAs affected only weakly, if not at all, the OR estimates. This suggests that BCAAs are at least in part responsible for the detrimental effects observed by diets rich in these components, such as the western diet.

In a study of colorectal cancer, we reported no association with BCAA intake, but BCAAs were related to a reduced risk of sigmoid cancer [23]. This confirms that nutritional risk factors for pancreatic cancer were at least in part different for those of colorectal cancer [3].

Limitations of the study are generic concerns about hospital-based case–control studies [41]. Selection bias cannot be excluded and dietary habits of hospital-controls may differ from the general population, but we excluded from the control group all subjects diagnosed with conditions associated with long-term dietary modifications. We had information on TNM on a limited number of cancers. Most of these were advanced cases. Thus, our study refers essentially to advanced pancreatic cancer, though there is no consistent evidence that risk factors are appreciably different for early versus late pancreatic cancer [3]. Among the strengths of the study is the large sample and the fact that cases and controls were from comparable catchment areas and interviewed in a similar setting. Moreover, the almost complete participation is reassuring in terms of potential selection bias. We used a satisfactorily reproducible and valid FFQ, with r values for BCAA food sources between 0.6 and 0.7 [31; 32]. Dietary information refers to the habitual diet in the years before the diagnosis or hospital admission, excluding bias due to reverse causation. Recall bias can be influenced by recent diagnosis of cancer but remains unlikely, given the scanty knowledge by the Italian population on a link between diet and pancreatic cancer risk at the time of information collection.

We controlled for major confounders, including smoking, BMI and diabetes. All participants were Caucasian; thus, race/ethnicity cannot confound results in this study. We were not able to adjust for chronic pancreatitis, which is a known risk factor for pancreatic cancer, but it accounts for a minor proportion of cases only [3]. Lack of blood samples can be another limitation, as we were not able to estimate the variability between BCAAs from diet and circulating levels of BCAAs, and to compare their effects on pancreatic cancer risk estimates.

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The observed association was consistent in sensitivity analyses excluding in turn subjects with diabetes, family history of pancreatic cancer and outliers in energy intake. In addition, the association was consistent across strata of several covariates, though possibly stronger in heavy alcohol drinkers and tobacco smokers. The combination of high BCAA intake with heavy smoking or heavy drinking is compatible with a multiplicative or supra-multiplicative effect of the two exposures, leading to excessively high ORs for subjects heavily exposed to smoking or alcohol in addition to BCAAs.

Taking into account the still rising rates and fatality of this aggressive cancer, and the absence of non-invasive screening tools to date [3], approaches to improve primary prevention and early diagnosis, such as dietary guidelines or tests based on specific metabolites, are warranted. Our results on BCAAs point in that direction, but they need further confirmation.

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Authorship:

Conception and design: MR and CLV; Collection of data: MF, DS, EN and CLV; Analysis of data: MR, FT and PS; Drafting the manuscript: MR and PS. All authors contributed to data interpretation, critical revision and final approval of the manuscript.

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Bibliography

1. Sung H, Ferlay J, Siegel RL et al. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin.

2. Santucci C, Carioli G, Bertuccio P et al. (2020) Progress in cancer mortality, incidence, and survival: a global overview. Eur J Cancer Prev 29, 367-381.

3. Kleeff J, Korc M, Apte M et al. (2016) Pancreatic cancer. Nat Rev Dis Primers 2, 16022.

4. Chen X, Yi B, Liu Z et al. (2020) Global, regional and national burden of pancreatic cancer, 1990 to 2017: Results from the Global Burden of Disease Study 2017. Pancreatology 20, 462-469.

5. Rosato V, Polesel J, Bosetti C et al. (2015) Population attributable risk for pancreatic cancer in Northern Italy. Pancreas 44, 216-220.

6. Lucenteforte E, La Vecchia C, Silverman D et al. (2012) Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Ann Oncol 23, 374-382.

7. Tramacere I, Scotti L, Jenab M et al. (2010) Alcohol drinking and pancreatic cancer risk: a meta-analysis of the dose-risk relation. Int J Cancer 126, 1474-1486.

8. Mizrahi JD, Surana R, Valle JW et al. (2020) Pancreatic cancer. Lancet 395, 2008-2020.

9. Rossi M, Negri E, Bosetti C et al. (2008) Mediterranean diet in relation to body mass index and waist-to-hip ratio. Public Health Nutr 11, 214-217.

10. Sivanand S, Vander Heiden MG (2020) Emerging Roles for Branched-Chain Amino Acid Metabolism in Cancer. Cancer Cell 37, 147-156.

11. Agnoli C, Sieri S, Ricceri F et al. (2018) Adherence to a Mediterranean diet and long-term changes in weight and waist circumference in the EPIC-Italy cohort. Nutr Diabetes 8, 22.

12. Rossi M, Turati F, Lagiou P et al. (2013) Mediterranean diet and glycaemic load in relation to incidence of type 2 diabetes: results from the Greek cohort of the population-based European Prospective Investigation into Cancer and Nutrition (EPIC). Diabetologia 56, 2405-2413.

Accepted manuscript

13. Schlesinger S, Neuenschwander M, Schwedhelm C et al. (2019) Food Groups and Risk of Overweight, Obesity, and Weight Gain: A Systematic Review and Dose-Response Meta-Analysis of Prospective Studies. Adv Nutr 10, 205-218.

14. Zheng J, Guinter MA, Merchant AT et al. (2017) Dietary patterns and risk of pancreatic cancer: a systematic review. Nutr Rev 75, 883-908.

15. Polesel J, Talamini R, Negri E et al. (2010) Dietary habits and risk of pancreatic cancer: an Italian case-control study. Cancer Causes Control 21, 493-500.

16. Di Maso M, Talamini R, Bosetti C et al. (2013) Red meat and cancer risk in a network of case-control studies focusing on cooking practices. Ann Oncol 24, 3107-3112.

17. de la OV, Zazpe I, Ruiz-Canela M (2020) Effect of branched-chain amino acid supplementation, dietary intake and circulating levels in cardiometabolic diseases: an updated review. Curr Opin Clin Nutr Metab Care 23, 35-50.

18. McCormack SE, Shaham O, McCarthy MA et al. (2013) Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. Pediatr Obes 8, 52-61.

19. Newgard CB, An J, Bain JR et al. (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 9, 311-326.

20. Guasch-Ferre M, Hruby A, Toledo E et al. (2016) Metabolomics in Prediabetes and Diabetes: A Systematic Review and Meta-analysis. Diabetes Care 39, 833-846.

21. Tobias DK, Clish C, Mora S et al. (2018) Dietary Intakes and Circulating Concentrations of Branched-Chain Amino Acids in Relation to Incident Type 2 Diabetes Risk Among High-Risk Women with a History of Gestational Diabetes Mellitus. Clin Chem 64, 1203-1210.

22. Zheng Y, Li Y, Qi Q et al. (2016) Cumulative consumption of branched-chain amino acids and incidence of type 2 diabetes. Int J Epidemiol 45, 1482-1492.

23. Rossi M, Mascaretti F, Parpinel M et al. (2020) Dietary intake of branched-chain amino acids and colorectal cancer risk. Br J Nutr, 1-6.

24. Tobias DK, Hazra A, Lawler PR et al. (2020) Circulating branched-chain amino acids and long-term risk of obesity-related cancers in women. Sci Rep 10, 16534.

25. Zeleznik OA, Balasubramanian R, Ren Y et al. (2020) Branched chain amino acids and risk of breast cancer. medRxiv, 2020.2008.2031.20185470.

26. Katagiri R, Goto A, Nakagawa T et al. (2018) Increased Levels of Branched-Chain Amino Acid Associated With Increased Risk of Pancreatic Cancer in a Prospective Case-Control Study of a Large Cohort. Gastroenterology 155, 1474-1482 e1471.

27. Mayers JR, Wu C, Clish CB et al. (2014) Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. Nat Med 20, 1193-1198.

28. Lee JH, Cho YR, Kim JH et al. (2019) Branched-chain amino acids sustain pancreatic cancer growth by regulating lipid metabolism. Exp Mol Med 51, 1-11.

29. Liu KA, Lashinger LM, Rasmussen AJ et al. (2014) Leucine supplementation differentially enhances pancreatic cancer growth in lean and overweight mice. Cancer Metab 2, 6.

30. Li JT, Yin M, Wang D et al. (2020) BCAT2-mediated BCAA catabolism is critical for development of pancreatic ductal adenocarcinoma. Nat Cell Biol 22, 167-174.

31. Franceschi S, Negri E, Salvini S et al. (1993) Reproducibility of an Italian food frequency questionnaire for cancer studies: results for specific food items. Eur J Cancer 29A, 2298-2305.

32. Decarli A, Franceschi S, Ferraroni M et al. (1996) Validation of a food-frequency questionnaire to assess dietary intakes in cancer studies in Italy. Results for specific nutrients. Ann Epidemiol 6, 110-118.

33. Gnagnarella P, Parpinel M, Salvini S et al. (2004) The update of the Italian Food Composition Database. J Food Compos Anal 17, 509-522.

34. Bravi F, Polesel J, Bosetti C et al. (2011) Dietary intake of selected micronutrients and the risk of pancreatic cancer: an Italian case-control study. Ann Oncol 22, 202-206.

35. Willett W, Stampfer MJ (1986) Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 124, 17-27.

36. Research. WCRFAIfC (2018) Diet, nutrition, physical activity and pancreatic cancer. Continuous Update Project Expert Report Available at dietandcancerreport.org.

37. Iwasaki M, Ishihara J, Takachi R et al. (2016) Validity of a Self-Administered Food-Frequency Questionnaire for Assessing Amino Acid Intake in Japan: Comparison With Intake From 4-Day Weighed Dietary Records and Plasma Levels. J Epidemiol 26, 36-44.

38. Chan JM, Wang F, Holly EA (2007) Pancreatic cancer, animal protein and dietary fat in a population-based study, San Francisco Bay Area, California. Cancer Causes Control 18, 1153-1167.

39. Bosetti C, Turati F, Dal Pont A et al. (2013) The role of Mediterranean diet on the risk of pancreatic cancer. Br J Cancer 109, 1360-1366.

40. Chan JM, Gong Z, Holly EA et al. (2013) Dietary patterns and risk of pancreatic cancer in a large population-based case-control study in the San Francisco Bay Area. Nutr Cancer 65, 157-164.

41. Breslow NE DN (1980) Statistical methods in cancer research. vol 1. The analysis of case–control studies. Lyon: IARC.

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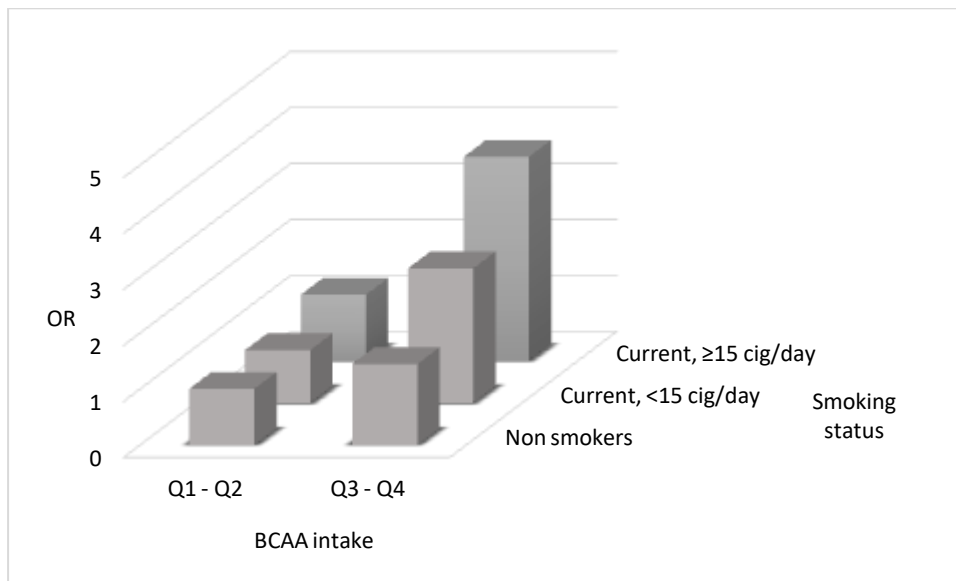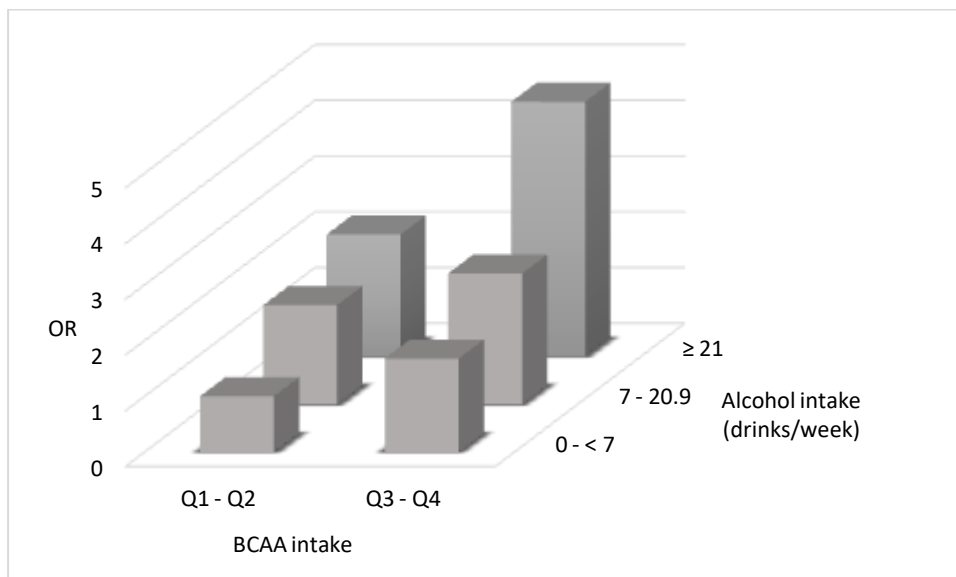
Panel A

Panel B

Figure 1 Odd ratios (ORs)[*] and corresponding 95% confidence intervals (CI) according to combination of quartiles[†] of branched-chain amino acid (BCAA) intake and smoking status (Panel A) or alcohol consumption (Panel B) among 326 cases with pancreatic cancer and 652 controls. Italy, 1991-2008

[*] Estimated through logistic regression models, conditioned on age, center, sex, and adjusted for year of interview, education, family history of pancreatic cancer, body mass index, diabetes, smoking, alcohol and total energy intake, unless the variable was the stratification factor.

[†] Control generated quartiles;

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Table 1. Distribution of 326 patients with pancreatic cancer and 652 control patients according to age, education and other selected variables (Italy, 1991-2008).

Characteristic	Cases		Controls	
	No.	%	No.	%
Sex				
Men	174	53.4	348	53.4
Women	152	46.6	304	46.6
Age (years)				
<50	32	9.8	64	9.8
50-59	89	27.3	178	27.3
60-69	122	37.4	244	37.4
≥70	83	25.5	166	25.5
Centre				
Milan	151	46.3	302	46.3
Aviano/Pordenone	175	53.7	350	53.7
Year of interview				
1991-1999	126	38.7	472	72.4
2000-2008	200	61.3	180	27.6
Education (years)[*]				
<7	166	51.2	350	53.9
7-12	86	26.5	192	29.5
≥12	72	22.2	108	16.6
Body mass index (kg/m^2)[*]				
< 25	139	42.9	264	40.6
≥ 25	185	57.1	385	59.4
History of diabetes[†]				
No	279	85.6	615	94.3
Yes	47	14.4	37	5.7
Family history of pancreatic cancer				
No	316	96.9	637	97.7
Yes	10	3.1	15	2.3
Smoking status[*]				
Non smokers	223	69.0	517	79.7
Current smokers	100	31.0	132	20.3
Alcohol consumption (drinks/week)[*]				
< 7	87	27.0	219	33.7
7-20.9	110	34.2	223	34.3
≥21	125	38.8	208	32.0

[*] The sum does not add up to the total because of some missing values.

[†] History of diabetes one year before cancer diagnosis.

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Table 2 Odds ratios (ORs)[*] and corresponding 95% confidence intervals (CIs) according to quartiles[†] of branched-chain amino acid (BCAA) intakes among 326 cases with pancreatic cancer and 652 controls (Italy, 1991-2008).

BCAA intakes	Upper cut-off (g/d).	Cases (%)	Controls (%)	OR (95% CI)
I	12.1	61 (19.0)	163 (25.0)	1 (reference)
II	14.7	73 (22.4)	163 (25.0)	1.23 (0.77-1.98)
III	17.9	86 (26.4)	163 (25.0)	1.88 (1.08-3.26)
IV	-	105(32.2)	163 (25.0)	2.17 (1.17-4.06)
χ^2 **trend (p-value)**				6.6 (0.010)

[*] Estimated through a logistic regression model, conditioned on age, center and sex, and adjusted for year of interview, education, , body mass index, diabetes, family history of pancreatic cancer, smoking, alcohol, and total energy intake;

[†] Based on the controls' distribution.

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Table 3 Odds ratios (ORs)[*] and corresponding 95% confidence intervals (CIs) according to quartiles[†] of branched-chain amino acid (BCAA) intake among 326 cases with pancreatic cancer and 652 controls after adjustment of selected dietary factors (Italy. 1991-2008).

Further adjustment for intake of:	OR (95% CI) Quartile of BCAA intake				χ^2 trend (p-value)
	1[‡]	2	3	4	
vegetable protein	1	1.24	1.90	2.22	6.69
		(0.77-1.99)	(1.09-3.31)	(1.18-4.17)	0.001
Total lipids	1	1.24	1.86	2.04	5.09
		(0.76-2.01)	(1.04-3.32)	(1.06-3.92)	<0.001
fiber	1	1.26	1.95	2.34	7.69
		(0.79-2.03)	(1.12-3.40)	(1.25-4.40)	0.006
folate	1	1.27	1.98	2.43	7.73
		(0.76-2.04)	(1.12-3.48)	(1.26-4.68)	0.006
vitamin E	1	1.29	1.96	2.29	7.27
		(0.80-2.08)	(1.12-3.43)	(1.22-4.30)	0.007
vitamin D	1	1.26	1.91	2.17	5.69
		(0.77-2.04)	(1.06-3.41)	(1.11-4.24)	0.017
calcium	1	1.22	1.90	2.28	5.97
		(0.74-1.98)	(1.04-3.44)	(1.14-4.58)	0.015
red meat	1	1.22	1.90	1.28	4.88
		(0.74-1.98)	(1.04-3.44)	(1.14-4.58)	0.027

[*] Estimated through a logistic regression model, conditioned on age, center and sex, and adjusted for year of interview, education, body mass index, diabetes, family history of pancreatic cancer, smoking, alcohol, total energy intake and in turn intake of each dietary factor;

[†] Control generated quartiles;

[‡] Reference category;

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Table 4 Odd ratios (ORs)[*] and corresponding 95% confidence intervals (CI) for quartiles[†] of branched-chain amino acid (BCAA) intakes among 326 cases with pancreatic cancer and 652 controls according to strata of selected covariates (Italy, 1991-2008).

BCAA quartiles	Covariates										
	Sex cases: controls		Age cases: controls		Body mass index (kg/m^2) cases: controls		Smoking status cases: controls		Alcohol consumption (drinks/week) cases: controls		
	Men 174:348	Women 152:304	< 60 121:242	≥ 60 205:410	< 25 139:264[‡]	≥ 25 185:385[‡]	Never/ex 223:517[‡]	Current 100:132[‡]	0 -< 7 87:219[‡]	7-20.9 110:223[‡]	≥21 125:208[‡]
I N.	21:63	41:100	18:50	44:113	40:74	22:88	48:133	14:30	26:68	23:53	12:40
OR (95% CI)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
II N.	29:83	44:80	26:56	47:107	33:72	39:91	54:126	18:36	22:65	28:49	23:49
OR (95% CI)	1.33 (0.61-2.88)	1.26 (0.67-2.36)	1.34 (0.53-3.39)	1.18 (0.67-2.06)	0.91 (0.45-1.84)	1.78 (0.87-3.64)	1.35 (0.78-2.31)	1.43 (0.48-4.30)	0.88 0.39-1.95	2.12 0.89-5.09	3.90 1.28-11.8
III N.	51:89	35:74	35:58	51:105	34:57	51:105	50:131	36:30	21:51	30:64	34:48

OR (95% CI)	2.40 (1.02-5.68)	1.66 (0.76-3.63)	3.87 (1.25-11.9)	1.37 (0.71-2.63)	1.90 (0.78-4.64)	2.36 (1.07-5.24)	1.46 (0.75-2.85)	7.12 (2.08-24.4)	1.26 0.47-3.34	3.58 1.21-10.6	4.79 1.44-15.9
IV N.	73:113	32:50	42:78	63:85	32:61	73:101	71:127	32:36	18:35	29:57	56:71
OR (95% CI)	2.85 (1.12-7.26)	1.88 (0.74-4.78)	3.94 (1.17-13.2)	2.11 (0.99-4.51)	1.40 (0.53-3.74)	3.64 (1.47-9.01)	1.95 (0.92-4.14)	5.24 (1.33-20.6)	1.44 0.43-4.81	3.77 1.09-13.1	7.39 2.09-26.2
p for heterogeneity	0.965		0.452		0.197		0.584			0.390	

[*] Estimated through logistic regression models, conditioned on age, center, sex, and adjusted for year of interview, education, family history of pancreatic cancer, body mass index, diabetes, smoking, alcohol and total energy intake, unless the variable was the stratification factor.

[†] Control generated quartiles;

[†] The sum does not add up to the total because of some missing values.