A PROSPECTIVE EVALUATION OF PANCREATIC CANCER RISK IN RELATION TO DIETARY ONE-CARBON METABOLISM-RELATED NUTRIENTS, SERUM B6 VITAMERS AND METABOLITES OF THE KYNURENINE PATHWAY

by

Yongxu Huang

BS, Tsinghua University, China, 2010

MPH, University of Pittsburgh, 2012

Submitted to the Graduate Faculty of

the Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2016

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This dissertation was presented

by

Yongxu Huang

It was defended on

September 2, 2016

and approved by

Lisa M Bodnar, PhD, MPH, RD
Associate Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Randall E. Brand, MD
Professor of Medicine, School of Medicine, University of Pittsburgh
Division of Gastroenterology, Hepatology and Nutrition
University of Pittsburgh Medical Center

Anna E. Lokshin, PhD
Professor of Medicine, Pathology, and Obstetrics, Gynecology, and Reproductive Sciences
University of Pittsburgh Cancer Institute

Jian-Min Yuan, MD, PhD
Professor, Arnold Palmer Endowed Chair in Cancer Prevention
University of Pittsburgh Cancer Institute
Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh

Dissertation Advisor: Lesley M. Butler, PhD
Associate Professor, Department of Epidemiology,
Graduate School of Public Health, University of Pittsburgh
University of Pittsburgh Cancer Institute

Lesley M. Butler, MSPH, PhD

A PROSPECTIVE EVALUATION OF PANCREATIC CANCER RISK IN RELATION TO DIETARY ONE-CARBON METABOLISM-RELATED NUTRIENTS, SERUM B6 VITAMERS AND METABOLITES OF THE KYNURENINE PATHWAY

Yongxu Huang, PhD

University of Pittsburgh, 2016

ABSTRACT

Pancreatic cancer is one of the most lethal human cancers. No effective long-term treatment is available and few risk factors have been identified for pancreatic cancer. Therefore, there is a critical need for identifying novel primary prevention targets. One-carbon metabolism-related nutrients such as vitamin B_6 and choline play an important role in DNA synthesis and methylation. The availability of methyl groups in the one-carbon metabolism are associated with epigenetic events related to pancreatic carcinogenesis. In the first prospective cohort study of Singapore Chinese (271 pancreatic cancer cases), we found higher intake of vitamin B_6 and choline were associated with reduced risk of pancreatic cancer. Compared with the lowest quartile, hazard ratios (HRs) and 95% confidence intervals (CIs) for the highest quartiles of vitamin B_6 and choline were 0.52 (0.36, 0.74) (P for trend = 0.001) and 0.67 (0.48, 0.93) (P for trend = 0.04), respectively. There were no clear associations between the other one-carbon metabolism-related nutrients and pancreatic cancer risk. To further investigate the role of vitamin B_6 in pancreatic cancer development, in the second case-control study nested within two prospective cohorts of Asian populations, a biomarker-based approach was used to evaluate the associations between B_6 vitamers in serum and risk of developing pancreatic cancer. The main finding of the second study was an inverse association between serum pyridoxal-5'-phosphate

(PLP), the active form of vitamin B_6, and pancreatic cancer risk. Compared with PLP deficient individuals (<20 nmol/L), the odds ratio (OR) and 95% confidence interval for PLP greater than 52.4 nmol/L was 0.46 (0.23, 0.92) (P for trend = 0.048). The inverse association between serum PLP and pancreatic cancer risk lends further support on dietary findings of vitamin B_6. In the third case-control study nested within the same two cohorts, we found higher ratios of metabolites of the kynurenine (Kyn) pathway, as biomarkers for intracellular functional status of PLP, were associated with reduced risk of pancreatic cancer. Compared with the lowest tertiles, the second and third tertiles of 3'-hydroxyanthranilic acid (HAA):3'-hydroxykynurenine (HK) ratio and HAA:Kyn ratio were associated with about 40% reduced risk of pancreatic cancer. In addition, we found that higher serum concentrations of HAA, an anti-inflammatory metabolite of the PLP-dependent Kyn pathway, were associated with reduced risk of pancreatic cancer. Compared with the lowest tertile, the ORs and 95%CIs for the second and third tertiles of HAA were 0.61 (0.38-0.97) and 0.63 (0.39-1.01), respectively (P for trend =0.04). In summary, the three studies suggested a protective role of vitamin B_6 in pancreatic cancer development. The finding on HAA sheds light on the potential protective effect of vitamin B_6 may be via one of the PLP-dependent metabolic pathways, such as the Kyn pathway. The specific mechanisms underlying the potential protective effect against pancreatic cancer warrant future studies. This research is relevant to public health because understanding the role of the inter-individual variability of dietary nutrients, such as vitamin B_6, in the development of cancer, may lead to identification of individuals at high risk for pancreatic cancer, and the development of cancer prevention strategies for reduction of cancer incidence and mortality.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

My journey towards this dissertation project started long ago. Losing a loved one to cancer in my childhood compelled me to search for strategies to improve cancer prevention and survival. I hope the findings of this study, the inverse associations between vitamin B_6, choline, and kynurenines and pancreatic cancer risk, can contribute to the understanding of pancreatic cancer etiology and inspire the development of chemoprevention methods.

First and foremost, I would like to thank my mentors Drs. Jian-Min Yuan and Lesley Butler. Drs. Yuan and Butler have led me into the world of epidemiology, guided me through the darkness and mist of scientific discovery, and helped me develop expertise in research and scientific writing. As my role models, Drs. Yuan and Butler have taught me diligence and encouraged me to think creatively. I would like to thank my doctoral committee members, Drs. Lisa Bodnar, Randall Brand, and Anna Lokshin, who generously shared their expertise with me and provided me tons of valuable insights and suggestions. I would like to thank my colleagues Renwei Wang for his tremendous help in biostatistics throughout my doctoral studies and Jennifer Adams-haduch who helped me process biospecimens used in the study. I would like to thank my collaborators, Øivind Midttun, Per M. Ueland, Arve Ulvik, and Aizhen Jin for their continuous support in helping me through each paper. I would like to thank the participants and local PIs of the Singapore Chinese Health Study and the Shanghai Cohort Study, Woon-Puay Koh and Yu-Tang Gao, who provided the data and biospecimens used in the study. I would like

to thank my professors at Graduate School of Public Health who taught me the intricacies of epidemiology and public health. I would like to thank the staff members of University of Pittsburgh Cancer Institute and Department of Epidemiology for all of their help, in particular, Brooke Spencer, Lori Smith, and Rachel Tusick. I would like to thank my close friends Orrin Fidelis and Collette Ncube Foust, who gave me tremendous help and support throughout my graduate school.

Finally, I would like to thank my family. I dedicate my dissertation to my mother, Yun Luo, who is always on my side, encourages me to follow my passions, and has taught me the values of love, genuineness, honesty, perseverance, and resilience. This dissertation work would not have been possible without the people who have helped me.

1.0 EPIDEMIOLOGY OF PANCREATIC CANCER

1.1 INCIDENCE AND MORTALITY

Worldwide patterns of pancreatic cancer

Pancreatic cancer is the 13th most common cancer in the world(1). There were 337,872 newly diagnosed cases in the year 2012, accounting for 2.4 percent of cancer cases overall(1). Pancreatic cancer is most often diagnosed at advanced stages and therefore is nearly always lethal. It is ranked as the 7th most common cause of cancer death, accounting for 4 percent of all cancer deaths(1).

Pancreatic cancer affects more men than women, with the age-standardized incidence rate of 4.9 per 100,000 person-years in men and 3.6 per 100,000 in women worldwide(1). Few sex-related hormone factors, if any, may play a role in the development of pancreatic cancer(2, 3). On the other hand, the prevalence of tobacco smoking, the only confirmed environmental cause of pancreatic cancer(4), is higher in men than in women(5), which may contribute to the sex differences in pancreatic cancer rates.

Pancreatic cancer is more common in developed countries, where the overall rates are almost 3-fold higher than in less developed countries(1). Globally, the age-standardized incidence rate ranged from 7~10 per 100,000 person-years in regions of Northern, Central and Eastern Europe and the U.S. to less than 1 per 100,000 in areas of Africa and West Asia(1). One

possible reason for these differences is the high prevalence of risk factors for pancreatic cancer, for example, obesity and diabetes, in developed countries(6). However, the international variation in rates of pancreatic cancer needs to be interpreted with caution, as in less developed countries many cases might not be identified or reported due to inaccurate diagnosis methods and inadequate medical delivery system(7).

Region-specific patterns of pancreatic cancer

The U.S.

In the U.S., pancreatic cancer is the 12th most common cancer and the third leading cause of cancer-related death, where in 2016, it was estimated that 221,450 men and 20,330 women died from pancreatic cancer (8). Pancreatic cancer represents about 3% of overall new cancer cases and 7% of cancer related-deaths (9). In the U.S., the median age at diagnosis of pancreatic cancer is 71, which is somewhat older compared to 61 for breast cancer and 66 for prostate cancer(10). Within the U.S., the age-standardized incidence is higher in men (14.4 per 100,000 person-years) than women (11.2 per 100,000 person-years)(11), and it is higher in Blacks (16.6 per 100,000 person-years) than Whites (12.3 per 100,000 person-years)(11). Pancreatic cancer is projected to become the second leading cause of cancer-related death by 2030 (12). The lifetime risk of pancreatic cancer for both men and women in the US is 1.5% (13).

Asia: China and Singapore

Despite having a low incidence, over 40% of pancreatic cancer cases and deaths occur in Asia(1). Due to improved life expectancy and urbanization, in some Asian countries with large aging populations such as China, the mortalities of a series chronic diseases including pancreatic cancer is on the rise(14, 15). Furthermore, the recent increases in the prevalence of several risk

factors of pancreatic cancer such as cigarette smoking, obesity, and diabetes are likely to contribute to a future rise in pancreatic cancer incidence in Asia(16, 17). In several Asian countries such as Singapore and Korea, pancreatic cancer mortality rates are already high and are increasing(17, 18).

Comparing incidence between the U.S., China, and Singapore

Table 1 shows age-standardized incidence of pancreatic cancer and survival data in selected national and/or ethnic groups by sex (1) . The incidence is higher in males than in females in all four national and/or ethnic groups. The incidence rates across the 4 national and/or ethnic groups are comparable for both sexes. The U.S. whites had the highest incidence rates for both sexes.

Incidence and Mortality Trends

A recent study analyzed the trends in pancreatic cancer mortality for 51 countries across the world for the period 1992-2002, and suggested that mortality rates were decreasing in the Western world (e.g. Canada, UK, and Switzerland) but increasing in Southern and Eastern Europe (e.g. Spain and Romania) and North East Asia (e.g. Korea and Japan) (19). Earlier studies showed pancreatic cancer incidence increased in industrialized countries such as Italy, France, and Japan in the period 1979-1998(20-22). In the U.S., pancreatic cancer incidence steadily decreased from 1973 to 2002 in men and increased until 1984 and then slowly decreased until 2002 in women(23). Given the rarity of pancreatic cancer in the general population, some of the changes in time trends could be due to chance. However, the rises in pancreatic cancer rates in some countries in Southern and Eastern Europe, and North East Asia may represent the recent increases in prevalence of tobacco smoking in these areas(24, 25). In contrast, countries in

Western Europe along with Canada and the U.S., the prevalence of smoking has been falling for decades(24).

Treatment and Survival

Pancreatic cancer has one of the lowest survival rate of all cancers, with a 5-year survival rate 8%(18) and a median survival of about 6 months(26). Surgical resection offers the best chance of survival (5 year survival rate: 20%) (27). However, due to a lack of early, specific symptoms and no effective screening test, only 15% of patients are diagnosed at an early stage when surgery is an option (27). Unlike with common cancers such as breast cancer(28) and prostate cancer(29) with 5-year survival rates of 89% and 99%, respectively, there has been no meaningful improvement in survival of pancreatic cancer over the last three decades(30). Several factors contributed to the poor prognosis of pancreatic cancer. First, metastasis happens in the early phase of the natural history of pancreatic cancer; second, as the disease progresses, patients usually present with severe morbidity such as cachexia (weakness and wasting of the body) and asthaenia (physical weakness and loss of strength); and third, pancreatic cancer responds poorly to most currently available treatments(31). Within the U.S., Asian and Pacific Islanders have better survival compared to the U.S. Whites, and similar observations were made by other studies(32). Shanghai Chinese have higher 5-year survival rates than in the U.S. Whites (**Table 1**). While pancreatic cancer patients in Shanghai may have longer survival than patients in the U.S., this difference is likely driven by the less rigorous standards for diagnosis in Shanghai than in Singapore and US.

1.2 RISK FACTORS

Below is a brief summary of the evidence for the most studied risk factors for pancreatic cancer, as shown in **Table 2**.

1.2.1 Established risk factors

Smoking

Current cigarette smoking is associated with a 77% increased risk of pancreatic cancer (33), and accounts for approximately 25% of pancreatic cancers(34). Carcinogens from tobacco may reach the pancreas through blood stream after absorption by lung or upper aerodigestive tract, and alternatively, or have direct contact with pancreas after reflux into pancreatic ductal system from the duodenum(35). Smoking may contribute to pancreatic carcinogenesis via induction of pro-inflammatory cytokines and chronic inflammation(36, 37), oxidative stress pathways, and fibrogenic mediators(38).

Obesity

Obesity is a recognized modifiable risk factor for pancreatic cancer. In general, three pooled studies(39-41) and two of three meta-analysis(42-44) showed positive associations between obesity and pancreatic cancer risk. In summary, every 5 kg/m^2 increase in body mass index (BMI) is associated with 10% or greater increase in pancreatic cancer risk. In other words, obese individuals ($BMI \geq 30$) have a 20 to 50% increases in pancreatic cancer risk compared to participants with normal BMI ($18.5 \leq BMI < 24.5$). A possible biological mechanism for the role of obesity in pancreatic cancer carcinogenesis is the hormonal and inflammatory effects of

adipose tissue. Adipose tissue is a metabolically active organ that secretes leptin and adiponectin, and cytokines (e.g. TNF-α and IL-6) that may regulate cell proliferation and tumor growth(45, 46).

Family history

Family history, defined as having at least one first-degree relative with pancreatic cancer, is associated with a relative risk between 1.7 and 5.0(47). A pooled analysis from Pancreatic Cancer Cohort Consortium (PanScan) found a history of prostate cancer increased pancreatic cancer risk by 45%(47). Germline genetic mutations are identified in 5-10% of pancreatic cancer cases(3). However, little is known about the specific genetic factors that cause pancreatic cancer. The most consistently identified common genetic factors are deleterious variants in *BRCA2* often leading to protein truncation (48, 49). BRCA2 carriers are associated with 3-10 fold increased risk of developing pancreatic cancer (50). The estimated cumulative risks of pancreatic cancer by age 80 in BRCA2 mutation carriers is 3.2% among males and 2.3% among females (51). Familiar atypical multiple mole melanoma syndrome (FAMMM) is an autosomally dominant disease with *p16/CDKN2A* gene mutation. The risk of developing pancreatic cancer is about 13-22 folds (50). In addition, individuals with Peutiz-Jeghers (PJ) syndrome, an autosomally dominantly disease caused by *STK11* gene mutations, had a 36% cumulatively lifetime risk of pancreatic cancer, and a relative risk of 132.

Chronic pancreatitis

Chronic pancreatitis is strongly associated with risk of pancreatic cancer. A meta-analysis of 6 cohort studies and one case-control study found chronic pancreatitis was associated with 13.3 times increased risk of pancreatic cancer (47). In *Kras*-mutated mouse models, both acute and chronic pancreatitis markedly accelerated the initiation and progression of pancreatic

cancer(52-54). The chronic pancreatitis-pancreatic cancer relationship provides evidence for an inflammatory component in pancreatic cancer development. Other than being a risk factor for pancreatic cancer *per se*, chronic pancreatitis shares many of the same risk factors for pancreatic cancer, such as alcohol, smoking, obesity, and diabetes(55), and thus, chronic pancreatitis may be an intermediate factor along the pancreatic carcinogenesis pathway(56-58).

Despite chronic pancreatitis is a strong risk factor for pancreatic cancer with biological plausibility, studies investigating the associations between chronic pancreatitis and pancreatic cancer could be subject to medical surveillance bias and the relative risks from those studies could be exaggerated. That is, chronic pancreatitis patients are more likely to go through clinical check-ups of pancreas compared to individuals without chronic pancreatitis. Therefore, subclinical pancreatic cancers are more likely to be detected among those with chronic pancreatitis than those without the disease. Evidence of medical surveillance bias could be found by comparing the frequency of clinical visits or the stage of pancreatic cancer between pancreatic cancer cases with a history of chronic pancreatitis and those without. Higher frequency of clinical visits and higher proportion of early stage diseases among pancreatic cancer cases with a history of chronic pancreatitis indicate medical surveillance bias. However, none of the studies investigating chronic pancreatitis-pancreatic cancer association has reported the information of frequency of clinical visits and pancreatic cancer stage at diagnosis (58-64).

In the meta-analysis conducted by Raimondi and colleges (47), the odds ratio of chronic pancreatitis in relation to pancreatic cancer risk dropped to 5.8 after excluding pancreatic cancer cases diagnosed within the first two years of a diagnosis of chronic pancreatitis, suggesting misdiagnosis of chronic pancreatitis for pancreatic cancer may inflate the odds ratio of 13 for pancreatic cancer associated with chronic pancreatitis.

1.2.2 Factors with limited or inconsistent evidence

Diet

The most extensively studied dietary nutrients in relation to pancreatic cancer risk are folate and vitamin D. A recent pooled analysis of 14 prospective cohort studies failed to find an association between folate intake and pancreatic cancer risk(65). Epidemiological studies observed mixed results on the associations between vitamin D intake or circulating 25-hydroxy vitamin D (a biomarker of vitamin D) and pancreatic cancer risk(66). A nested case-control study involving 365 pancreatic cancer cases over an average 16-year follow-up period suggested that dietary vitamin D was inversely associated with pancreatic cancer risk(67). However, a recent pooled analysis of 14 prospective cohort studies involving 2212 pancreatic cancer cases found no association between dietary vitamin D and pancreatic cancer risk(68).

The associations between myriad foods and pancreatic cancer have been extensively studied in epidemiological studies, but results are inconsistent on which foods are most relevant in the development of pancreatic cancer. Diets high in total fat are associated with increased risk of pancreatic cancer(35) but this has not been consistently reported (69). In general, epidemiological studies found pancreatic cancer risk increased with higher meat intake, especially fried and grilled meats and preserved meats that contain mutagenic and carcinogenic compounds, such as heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAHs), and *N*-nitroso compounds, respectively (46, 70-73). Most case-control studies have reported a weak to modest inverse association between total fruit and vegetable consumption and pancreatic cancer risk(74). However, prospective cohort studies generally found no association between total fruit and vegetable consumption and pancreatic cancer risk(75, 76).

One of the difficulties in assessing the association between diet and pancreatic cancer risk is the rapid clinical course of the disease. Early studies that assessed consumption of different type of foods and pancreatic cancer associations mainly used a retrospective case-control study design(35). Due to the severity of the disease, at the time of case ascertainment, most patients are unable to participate in the study and respond to detailed dietary questionnaires(35). The high case fatality of pancreatic cancer resulted in inclusion of only patients with relatively longer survival into case-control studies or heavy reliance on the surrogate data or both(77). In some studies, up to 88% of the case information is provided by a proxy interview (78). Therefore, to study the roles of dietary components in pancreatic cancer development, prospective designed studies seem to be more interpretable than retrospective case-control studies in terms of less survival bias and information bias.

Most studies that assessed the association between diet and pancreatic cancer risk were conducted in Western populations. Due to different dietary habits, the findings from those studies may be applicable to other populations. For example, compared to Western diet, Asian diet is higher in intakes of vegetables, fruit, and fish and lower in intakes of total energy, fat, sugar, red meat, and other animal products(79, 80). Pancreatic cancer rates are lower in Asian populations compared to populations in Europe and North America(1). The international variations in diet and pancreatic cancer rates continue to suggest that diet may be an important risk factor for pancreatic cancer. Pancreatic cancer may be partially preventable through modification of diet. Therefore, prospectively designed studies in other populations such as Asians will shed light on the roles of dietary components in pancreatic cancer development(81).

Alcohol

The associations between alcohol and pancreatic cancer risk are inconsistent across the spectrum of alcohol intake level (7). For example, a pooled analysis of 14 cohort studies with 2,187 cases found alcohol intake was positively associated with pancreatic cancer risk (82). Comparing individuals who drink more than 30 grams of alcohol per day to nondrinkers, the relative risk and 95% confidence interval are 1.22 (1.03, 1.45)(82). However, another pooled analysis of 12 nested case-control studies with 1,530 cases found no association between alcohol consumption and pancreatic cancer risk(83). The two pooled analysis studies shared only 4 cohorts, and the other cohorts are different. Although the effect of alcohol on pancreatic cancer risk is often confounded by smoking, one study found heavy alcohol consumption (≥3 drinks per day) was associated with a 36% increase in pancreatic cancer among never smokers excluding the possibility of residual confounding from smoking(84). At high level, alcohol may promote the effect of other risk factors such as tobacco smoking and inflammatory pathways involved in chronic pancreatitis(85). Additionally, alcohol may promote pancreatic cancer via increasing reactive oxygen species inducing oxidative DNA damage and interfering with one-carbon metabolism resulting in abnormal DNA methylation(85).

Physical activity

Prospective studies support inverse associations between high levels of several types of physical activity and pancreatic cancer risk(86). A prospective cohort study including 350 pancreatic cancer cases found an inverse association between moderate physical activity and pancreatic cancer risk. Comparing the highest to the lowest category [≥11 Metabolic Equivalent Tasks (METs) vs. < 0.9 METs for men, and ≥ 10.8 METs vs. < 0.8 METs for women], the

relative risk (RR) and 95% confidence interval (CI) are 0.45 (0.29, 0.70)(87). Physical activity may reduce pancreatic cancer risk via reducing insulin resistance or obesity(86).

1.3 ONE-CARBON METABOLISM

One carbon metabolism is a network of interrelated biochemical reactions, with folate and methionine as the key components (Figure 1). After ingestion, dietary folate goes through several reactions catalyzed by enzymes that require vitamin B2 and B6 as cofactors, supplying one carbon units for DNA synthesis and repair, and is ultimately converted to 5-methyl-tetrahydrofolate (5-MTHF) (88) (89). Methionine forms S-adenosylmethionine, a universal methyl donor in DNA, RNA, and protein methylations, and is eventually converted to homocysteine (90) (91). Methionine can be replenished via remethylation of homocysteine, either catalyzed by methionine synthase (MTR) that requires vitamin B12 and 5-MTHF, or catalyzed by betaine homocysteine methyltransferase (BHMT) that requires betaine, which can be ingested or derived from choline (88). Alternatively, homocysteine can be converted to glutathione, an important antioxidant (92). Vitamin B_6 is a cofactor for multiple enzymes in the one-carbon metabolism pathway, including serine hydroxymethyltransferase for nucleotide synthesis (93) and remethylation of homocysteine (94), and cystathionine β-synthase and cystathione γ-lyase for generation of glutathione (95).

One carbon metabolism has been implicated in cancer etiology due to its roles in DNA synthesis, repair, and methylation. Pancreatic cancer shows features of frequent genetic alterations (96) and abnormal DNA methylation patterns(97, 98), and thus the availability of one carbon metabolism-related nutrients may affect pancreatic cancer risk. Experimental studies

provided evidence that abnormal one carbon metabolism may affect pancreatic cancer initiation and growth. For example, vitamin B_6 deficient diet induced DNA damage in the pancreas in rats (99), choline-deficient diet enhanced the development of carcinogen-initiated pancreatic cancer in rodents (100), and L-methionine inhibited growth of human pancreatic cancer cell lines through inducing apoptosis (90).

Epidemiological studies showed inconsistent results on the associations between dietary intake of one carbon metabolism-related nutrients and pancreatic cancer. Although a 2007 report by World Cancer Research Fund suggested that folate from food may have a probable protective effect on pancreatic cancer development (10). A recent pooled analysis of 14 prospective cohort studies found pancreatic cancer was not associated with dietary or total (dietary plus supplemental) intake of folate (65). In contrast, a recent meta-analysis of 4 case-control studies and 6 prospective cohort studies observed inverse association between dietary folate intake and pancreatic cancer risk (101). However, the meta-analysis reported statistically significant heterogeneity across studies included in the analysis and significant publication bias. In contrast to the enormity of research efforts on folate-pancreatic cancer association, less attention has been drawn to other nutrients that participate into one carbon metabolism. Among the two prospective cohort studies that assessed methionine intake and pancreatic cancer risk, one reported inverse association (102), and another one reported no association (103). Two prospective cohort studies reported no association between vitamin B_6 intake and pancreatic cancer (102, 103).

The conflicting study results may be partly because of the limited number of nutrients assessed in some studies. Due to the complexity of one carbon metabolism, a comprehensive assessment of multiple nutrients may be more likely to reflect the overall nutrient status of one carbon metabolism and allow identification of the most critical nutrient in relation to pancreatic

cancer development. Although numerous studies were conducted in Western populations, to our knowledge no studies were conducted in Asian populations. Associations between dietary one carbon metabolism-related nutrients and pancreatic cancer observed in Western populations may not apply to Asian populations, given the different dietary habits and food sources of one carbon metabolism-related nutrients in Asian versus western populations. For example, The main food sources of vitamin B6 in the US population are ready-to-eat cereal (14.6%), beef (9.6%), poultry (9.6%), and potato (8.9%) (104). In the Singapore Chinese Health Study, the main sources of vitamin B_6 are grain products (24.5%), fish and shellfish (15.7%), fruits and related juice (14.8%), vegetable and related juice (12.6%), and red meat (8%).

To address these limitations, in the first study, we analyzed data from a prospective cancer epidemiology cohort in Singapore Chinese to test the hypothesis that high intakes of one carbon metabolism-related nutrients are inversely associated with the development of pancreatic cancer.

1.4 B_6 VITAMERS

Vitamin B_6 comes from a large number of foods. The richest food sources of vitamin B_6 include beef liver and other organ meats, fish, potato and other starchy vegetables, and non-citrus fruits (104-106). PLP is the bioactive form of vitamin B_6 that serves as an enzymatic cofactor in metabolisms of amino acids, one-carbon units, neurotransmitters, and immunomodulatory metabolites(107). Circulating level of PLP is used as a primary index of whole-body vitamin B_6 availability(108). To examine closely the role of internal dose or whole-body availability of

vitamin B_6, a biomarker approach is needed to evaluate the association between vitamin B_6 and risk of pancreatic cancer.

Three studies have evaluated the association between circulating levels of PLP and pancreatic cancer risk. Two case-control studies nested within prospective cohorts of European and the U.S. populations found no association between plasma PLP and pancreatic cancer risk(109, 110). Another case-control study nested among a cohort of Finnish male smokers found higher serum PLP was associated with a decreasing trend in pancreatic cancer risk(111). The highest tertile of serum PLP was associated with a 50% reduction in pancreatic cancer risk compared to the lowest tertile(111). It has been established that smokers have lower levels of plasma PLP, which may limit the generalizability of the study results to nonsmokers(112). An investigation of circulating PLP and pancreatic cancer risk in populations other than the U.S. or Europe is needed given the different levels of vitamin B_6 exposure across populations worldwide. In addition, previous studies only focused on PLP. Other major forms of B_6 vitamers in circulation may provide a more relevant measure of available vitamin B_6 than evaluating PLP alone in relation to pancreatic cancer risk. Therefore, to fill the gap in the literature and have more understanding of the association between vitamin B_6 vitamers and pancreatic cancer risk, the second study used a nested case-control study design and comprehensively evaluated the role of serum B_6 vitamers in relation to pancreatic cancer risk using the data from two prospective cohort studies in Asian populations.

In addition to PLP, pyridoxal (PL) and 4-pyridoxic acid (PA) are major forms of B_6 vitamers in circulation (112) (Figure 2). PL is the transport form of vitamin B_6 that crosses cellular membranes, which can be interconverted with PLP(113). PA is the predominant catabolite of PLP in excretion(113). In a human feeding trial, the circulating levels of PLP, PL,

and PA were all elevated in respond to vitamin B_6 supplementation, indicating PL and PA are valid biomarkers for vitamin B_6 status(114). Therefore, the sum of PLP, PL, and PA can be used as a marker of total PLP in circulation. Recently, the ratio of PA:(PL +PLP), or PAr has been proposed as a biomarker for catabolism of vitamin B_6(115) during the chronic inflammatory state(115). PAr is a strong indicator of chronic inflammation, suggesting increased catabolism may in part account for low PLP concentration in inflammatory conditions(107, 116, 117). In addition, high PAr is associated with increased oxidative stress(118), which is implicated in genomic instability and carcinogenesis(119). Whether PAr, a marker of vitamin B_6 catabolism during inflammation, is associated with pancreatic cancer risk is unknown.

1.5 METABOLITES OF THE KYNURENINE PATHWAY

The initial step of the kynurenine (Kyn) pathway is the indoleamine 2,3-dioxygenase (IDO)-catalyzed conversion of tryptophan to Kyn (Figure 3). Kyn can be metabolized to kynurenic acid (KA) by kynurenine aminotransferase (KAT), or alternatively, be metabolized to 3-hydroxykynurenine (HK) by kynurenine 3-monooxygenase (KMO). HK can be further converted to xanthurenic acid (XA) by KAT, or be converted to 3-hydoxy-anthranillic acid (HAA) by kynureninase.

PLP is a cofactor for kynurenine aminotransferase (KAT) and kynureninase, two enzymes in the Kyn pathway (Figure 3). Thus, low PLP may interfere with the metabolism of Kyn and production of downstream metabolites. This hypothesis is supported by experimental data and dietary restriction of vitamin B_6 in humans, where the concentration of KA was lower and the concentration of HK was higher in plasma from men and women fed a vitamin B_6

restricted diet compared to control subjects (120-122). Recently, the substrate product ratios of KAT (i.e., KA:HK and XA:HK) and kynureninase (i.e., HK/HAA) have been identified as markers of intracellular functional status of vitamin B_6 (the functional availability of vitamin B_6 in tissues to carry out enzymatic reactions)(123). In other words, higher ratios of KA:HK, XA:HK, and HAA:HK indicate lower availability of vitamin B_6 to carry out the enzymatic reactions in the Kyn pathway.

Inflammation has been implicated in pancreatic cancer initiation and progression(124). Vitamin B_6 supplementation has been shown to reduce circulating levels of pro-inflammatory cytokines (i.e. TNF-α and IL-6) in humans(125). The immune system relies on PLP-dependent Kyn metabolism pathway to regulate an inflammatory response. Current evidence suggests that the inverse association between circulating PLP and chronic inflammatory biomarkers may be explained by the mobilization of PLP into active inflammatory sites for use by the Kyn metabolism pathway(107).

Metabolites of the Kyn pathway are known to have anti-inflammatory properties. For example, in a mouse model, Kyn, HK, and XA restricted Th2-dependent allergic airway inflammation(126), and Th2 immune response is hypothesized to promote pancreatic cancer growth(127). Furthermore, several kynurenines including Kyn, KA, and XA have been identified as endogenous ligands that can bind to and activate aryl hydrocarbon receptor (AHR)(128). AHR is a transcription factor. The activation of AHR is involved in immune responses and inhibiting inflammation(129-131). AHR is highly expressed in human pancreatic cancer tissues and activation of AHR inhibited the growth of human pancreatic cancer cell lines via induction of the cyclin-dependent kinase inhibitor p21, leading to cell cycle arrest and inhibition of cancer growth(132, 133). Indeed, clinical evidence suggested Kyn level in plasma was negatively

correlated with tumor size in pancreatic cancer patients(134). Therefore, this third study tested the hypothesis that lower serum levels of kynurenines are associated with higher risk of pancreatic cancer.

In addition to the anti-inflammatory effects of downstream kynurenines, the ratio of Kyn to tryptophan (KTR) has been suggested as a marker of cellular immune activation. The IDO enzyme that catalyzes the initial and rate-limiting step of tryptophan degradation through the Kyn pathway can be induced by cytokines such as interferon-γ (IFN- γ). IFN-γ is considered a Th1 cytokine that protects against tumor development and promotes tumor surveillance(135). In addition to promoting antitumor cellular immunity, IFN- γ directly inhibited pancreatic cancer cell growth via activation of caspase-1 mediated apoptosis(136). The association between circulating KTR, a marker of cellular immune activation, and pancreatic cancer risk has not been studied yet. In addition to KTR, neopterin is also a marker of cellular immunity activation. Neopterin is a metabolite of guanosine triphosphate (GTP) and is produced by macrophages upon stimulation of IFN- γ. Due to the short half-life of IFN-γ *in vivo*(137), the direct measurement of IFN- γ in circulation is often difficult, and therefore, KTR and neopterin can be used as markers of IFN- γ-related cellular immunity activation(138). The third study investigated the role of ratios of kynurenines, KTR and neopterin, markers of cellular immunity activation, in relation to pancreatic cancer risk.

1.6 TABLES

Table 1. Age standardized (world) incidence rates (ASR) (per 100,000 persons per year) and 5-year relative survival (%) of pancreatic cancer (ICD10, C25) by selected national and/or ethnic group, and sex, 2003-2007

	Singapore Chinese		Shanghai City Chinese		USA, SEER (18 registries): White		USA, SEER(18 registries): Asian and Pacific Islander	
	Males	Females	Males	Females	Males	Females	Males	Females
No. cases	487	408	2100	1901	16,887	16,810	1382	1457
ASR (W)[a]	6.3	4.2	7.0	5.3	8.1	6.0	6.0	4.8
5-year relative survival, %	3.2[b]	3.6[b]	8.4[c]	7.1[c]	4.2[d]	4.4[d]	5.0[d]	7.1[d]

[a] Age-standardized (world) incidence rates (per 100,000 person-years)
[b] Cases diagnosed in 1993–1997 followed-up until 2001 (All residents in Singapore)
[c] Cases diagnosed in 1992–1995 followed-up until 2000
[d] Cases diagnosed in 1988-2001, age 20+, SEER12(139)

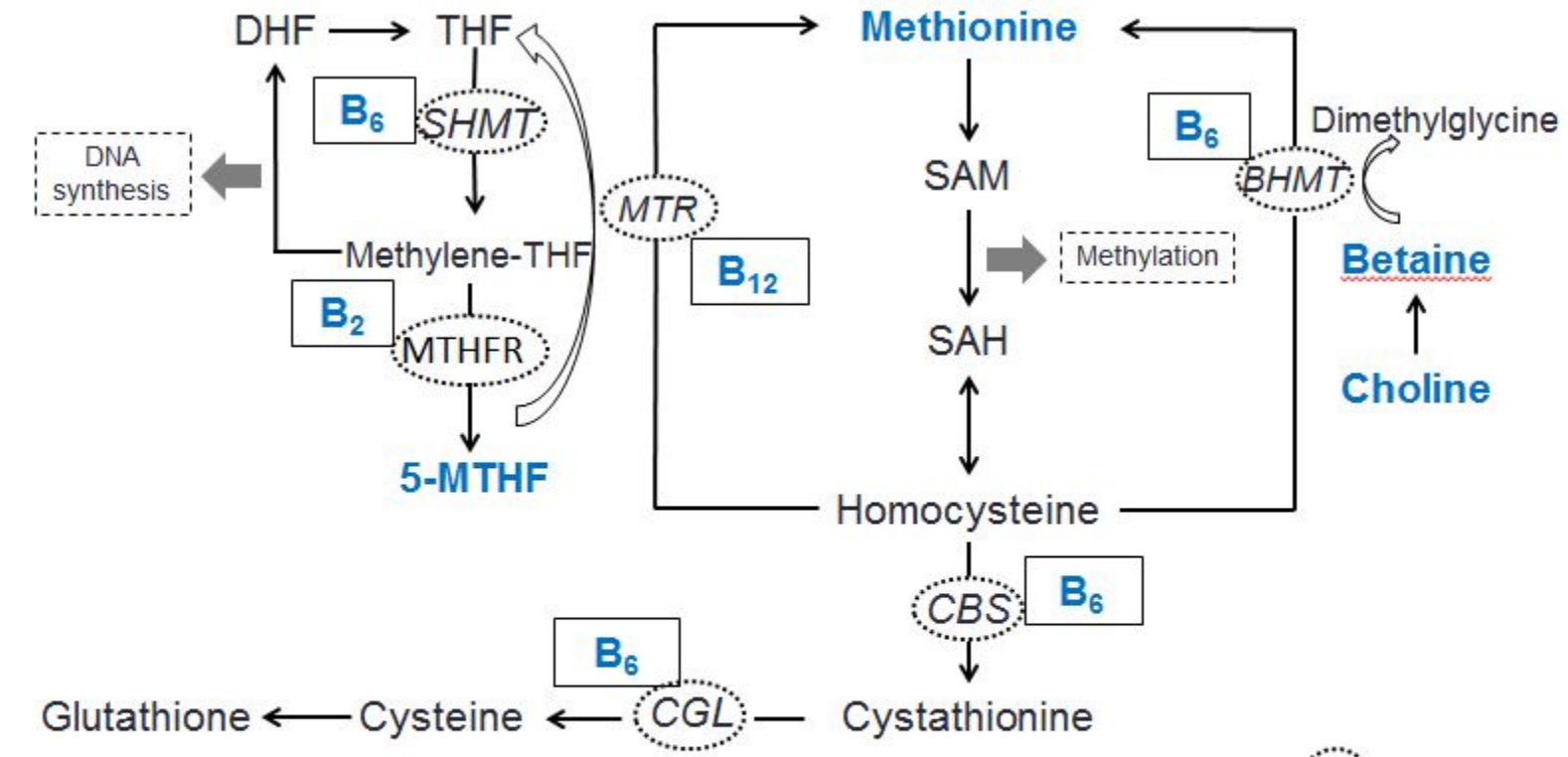
Table 2. Risk factors for pancreatic cancer

		Key Studies
Established risk factors	**Increase risk**	
	Smoking	A pooled analysis(33) from nested case-control studies involving 1,481 cases found for current smoker versus never smokers:
		OR=1.77, 95%CI:1.38, 2.26
	Obesity	A meta-analysis(42) of 6 case-control and 8 cohort studies involving 6,391 cases found that for per unit increase in BMI:
		OR=1.02, 95%CI:1.01, 1.03
	Family history	A pooled analysis(140) involving 1,183 cases found that for at least one first degree relative with pancreatic cancer versus no first degree relative with pancreatic cancer:
		OR=1.76, 95%CI:1.19, 2.61
	Chronic pancreatitis	A prospective cohort study(60) of 322 chronic pancreatitis patients over a median follow-up of 9 years found the age and sex standardized incidence ratio (SIR):
		SIR=19, CI: 5.2, 48.8
	Decrease risk	
	None	
Factors with limited or inconsistent evidence	**Increase risk**	
	Red meat	A meta-analysis(141) of 11 prospective studies with 6643 cases showed for an increase in red meat consumption of 120 g per day:
		RR=1.13, 95%CI: 0.93, 1.39
	Dietary fat	A prospective cohort study(142) involving 178 cases over 18 years of follow-up found for the 5^{th} quintile versus the 1^{st} quintile of total fat intake:
		RR=1.24, 95%CI:0.70, 2.20, p for trend=0.52
	Alcohol	A pooled analysis of 10 case-control studies(143) involving 5,585 cases showed that for people drinking ≥ 9 drinks per day:

Table 2 Continued

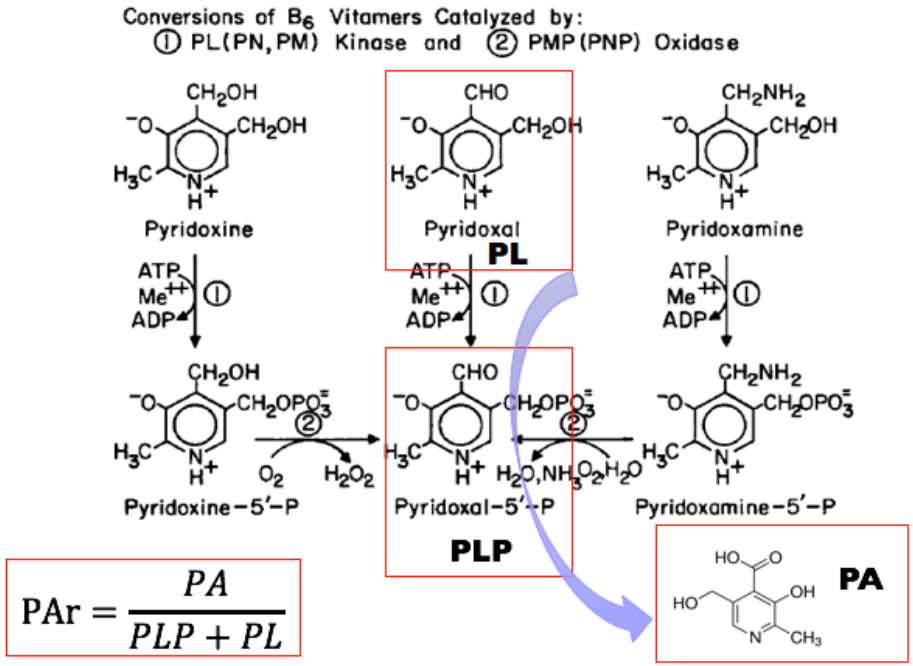
	OR=1.6, 95%CI: 1.2, 2.2
Decrease risk	
Fruits and vegetables	A large population-based case-control study(144) involving 532 cases found for the highest quartile versus the lowest quartile of total vegetable intake: OR=0.45, 95%CI: 0.32, 0.62, p for trend<0.0001 For the highest quartile versus the lowest quartile of total fruits and total fruit juice intake: OR=0.72, 95%CI: 0.54, 0.98, p for trend = 0.06
Folate	A pooled analysis of 14 prospective studies(65) involving 2,195 cases found that the highest versus the lowest quintile of dietary folate intake: RR=1.06, 95%CI: 0.90, 1.25, p for trend=0.47
Vitamin D	A pooled analysis of 14 prospective studies(68) involving 2,212 cases found that for dietary vitamin D ≥1300 IU versus <500 IU: HR=0.94, 95%CI: 0.64, 1.38, p for trend=0.12
Vitamin B_6	Two prospective cohort studies showed no association between dietary vitamin B_6 and pancreatic cancer risk. One Finnish nested case-control study involving 126 cases found inverse association between serum pyridoxal 5'-phosphate (PLP) and pancreatic cancer risk. For highest vs. lowest tertile: OR=0.48, 95%CI: 0.26, 0.88, p for trend = 0.02. Two other nested case-control studies conducted in European and US populations found no associations between serum PLP and pancreatic cancer risk.
Physical activity	A prospective study(87) involving 350 cases found for the highest versus the lowest category of moderate physical activity: RR=0.45, 95%CI=0.29, 0.70, p for trend < 0.001

1.7 FIGURES



Abbreviations: BHMT, betaine homocysteine methyltransferate; CBS, cystathionine β-synthase; CGL, cystathione γ-lyase; DHF, dihydrofolate; MTHF, methyl-tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

Figure 1. One-Carbon metabolism



Abbreviations: PL, pyridoxal; PN, pyridoxine; PM, pyridoxamine; PLP, pyridoxal-5'-phosphate; PNP, pyridoxine-5'-phosphate; PMP, pyridoxamine-5'-phosphate; 4-pyridoxic acid (4-PA).

Adapted from "Update on interconventions of vitamin B-6 with its coenzyme," by D. B. McCormick and H. Chen, 1999, The Journal of Nutrition, 129: 325-327, Copyright 2016 by the American Society for Nutrition.

Figure 2. Interconversions of B6 vitamers

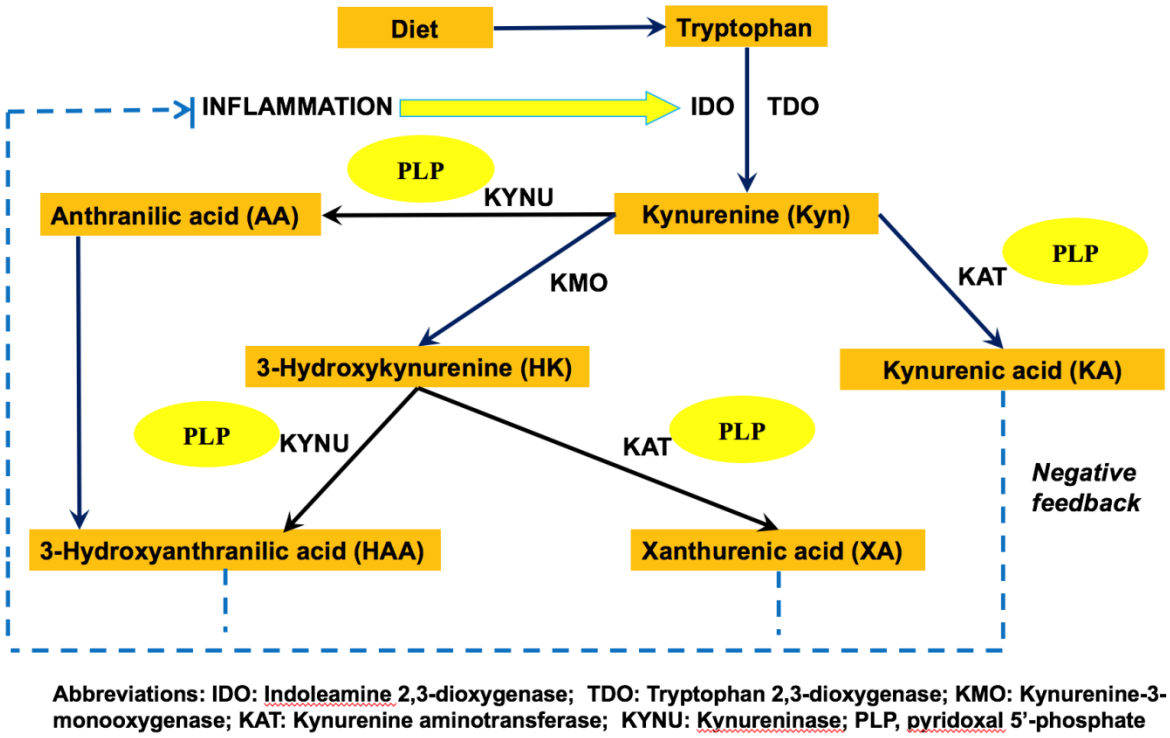


Figure 3. The kynurenine pathway

2.0 DIETARY INTAKE OF ONE-CARBON METABOLISM-RELATED NUTRIENTS AND PANCREATIC CANCER RISK: THE SINGAPORE CHINESE HEALTH STUDY

Background: Nutrients involved in one-carbon metabolism are hypothesized to protect against pancreatic cancer development. **Methods:** The Singapore Chinese Health Study database was used to prospectively examine the association between intake of one-carbon metabolism-related nutrients and pancreatic cancer risk. Between 1993 and 1998, 63,257 men and women aged 45-74 years were enrolled into the cohort. The daily intakes of the following one-carbon metabolism-related nutrients were assessed at enrollment using a 165-item food frequency questionnaire: betaine, choline, folate, and vitamins B_2, B_6, and B_{12}. Multivariable hazard ratios (HRs) and 95% confidence intervals (CIs) for pancreatic cancer risk associated with dietary intakes of one-carbon metabolism-related nutrients were calculated. **Results:** As of December 2013, 271 incident pancreatic cancer cases were identified during an average of 16.3 years of follow-up. Higher intake of vitamin B_6 and choline were associated with statistically significant decreases in the risk of developing pancreatic cancer. Compared with the lowest quartile, HRs (95% CIs) for the highest quartiles of vitamin B_6 and choline were 0.52 (0.36, 0.74) (*P* trend = 0.001) and 0.67 (0.48, 0.93) (*P* trend = 0.04), respectively. There were no clear associations between the other one-carbon metabolism-related nutrients and pancreatic cancer risk. **Conclusion:** Our study suggests that higher intake of vitamin B_6 and choline may lower the risk of pancreatic cancer. **Impact:** Our prospective findings are consistent with the *in vivo* evidence for protective roles of vitamin B_6 and choline on pancreatic cancer development.

2.1 INTRODUCTION

Pancreatic cancer is among the most deadly malignancies in the world. In the U.S., pancreatic cancer is the fourth leading cause of cancer-related death in both men and women (145). In 2014, it is estimated that 20,170 men and 19,420 women died from pancreatic cancer (145). The median survival of pancreatic cancer is only three to six months, due in part to the lack of effective treatments (26). Cigarette smoking and obesity are the only established modifiable risk factors for pancreatic cancer (146, 147). The identification of novel primary prevention targets is a viable approach for reducing the burden of pancreatic cancer.

One-carbon metabolism is a set of interconnected pathways that supply methyl groups for DNA synthesis, repair, and methylation (148). Adequate DNA methylation maintains chromosome stability and prevents gene disruption (149). Studies using a global methylation profiling approach showed that a substantial number of genes were aberrantly methylated not just in advanced pancreatic cancers (150), but also in precancerous lesions (151), indicating a potential time window when chemoprevention agents that target DNA methylation pathways could have a beneficial impact. Diet is a major source for key substrates and cofactors involved in one-carbon metabolism, such as vitamin B_6, choline, and folate (105). Vitamin B_6 is a cofactor for multiple enzymes in the one-carbon metabolism pathway, including serine hydroxymethyltransferase for nucleotide synthesis (93) and remethylation of homocysteine (94), and cystathionine β-synthase and cystathione γ-lyase for generation of glutathione (152). In rodents, diets deficient in methyl donors (i.e., choline, methionine, and/or folate) resulted in global hypomethylation (153-155) and increased incidence of neoplastic lesions induced by

carcinogens in the pancreas (100, 156, 157). Therefore, low dietary intake of these nutrients may interfere with the normal function of one-carbon metabolism pathways (120, 122), and thus increase the risk of developing pancreatic cancer.

A majority of epidemiologic studies that have evaluated one-carbon metabolism-related nutrients in relation to pancreatic cancer risk have focused on the potential role of folate. A recent pooled analysis of 14 prospective cohort studies conducted mostly in U.S. populations, reported that dietary folate was not associated with pancreatic cancer risk (65). Similarly, there was no trend with increasing blood folate levels and pancreatic cancer risk in the European Investigation of Cancer (EPIC) cohort study (110) or in a pooled analysis of U.S. cohorts (109).

Few prospective studies of pancreatic cancer have considered the potential role of one-carbon metabolism-related nutrients other than folate. Dietary methionine was strongly associated with lower risk of pancreatic cancer in a Swedish study (102), but not in a cohort of Finnish male smokers (103). While neither cohort supported dietary vitamin B_6 as a possible protective factor for pancreatic cancer (102, 103), a recent report showed a statistically inverse association for plasma pyridoxal phosphate (PLP), the bioactive form of vitamin B_6, in European women (110). A large population-based case-control study in the U.S. reported a positive association and trend with increasing pancreatic cancer risk for dietary vitamin B_{12} (158). There have been no epidemiologic studies of other one-carbon related nutrients, such as choline or betaine and pancreatic cancer risk.

Due to the involvement of multiple nutrients and the complexity of one-carbon metabolism pathways, a comprehensive assessment of the nutrients involved and their associations with pancreatic cancer risk is needed. Therefore, it is worthwhile to investigate these associations in a prospective cohort with well-characterized dietary intake. We analyzed data

from a prospective cohort of Singapore Chinese to test the hypothesis that higher intake of one-carbon metabolism-related nutrients are inversely associated with the risk of developing pancreatic cancer.

2.2 METHODS

2.2.1 Study design and population

The design of Singapore Chinese Health Study has been previously described in detail (159). Briefly, between 1993 and 1998, 63,257 Chinese men and women between ages of 45-74 years living in Singapore were enrolled into the cohort study. The cohort was drawn from residents of government-built housing estates (86% of all Singaporeans during the recruitment time period). Study subjects were restricted to two major dialect groups of Chinese in Singapore (Hokkien and Cantonese). The study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh.

2.2.2 Assessment of one-carbon metabolism-related nutrients

At the time of recruitment, each participant completed an in-person interview with a structured questionnaire asking for information on demographics, uses of tobacco and alcohol, medical history, and physical activity. To assess usual dietary intake, a 165-item semi-quantitative food frequency questionnaire (FFQ) was used. The FFQ was developed for the

target study population and validated using a series of 24-hour recall interviews and repeated administration of the FFQ in a subpopulation of the cohort (160).

Average daily intake of one-carbon metabolism-related nutrients was calculated by multiplying the usual frequency and portion size of each food item by the nutrient content using the food composition values from the Singapore Food Composition Database. The method is described as follows: sum up the intake of a certain nutrient from all 849 raw and cooked food items included in the Singapore Food Composition Database, which can be expressed as$\sum_i AiBi$. Here, Ai denotes the average daily consumption of a single food item i by grams, Bi denotes the level of a certain nutrient per 100 g edible food item i. For each food item, the weight Ai was estimated by usual frequency and portion size collected from the questionnaire, and corresponding weight for single units of all measurements. The nutrient contents information Bi were obtained from the original Singapore Food Composition Database, which was built upon a large unpublished data set developed at the Cancer Research Center of Hawaii (relied heavily on data published by the US Department of Agriculture (161, 162)), and supplemented with several published food composition tables of the People's Republic of China (163), Malaysia(164), and Taiwan (165). The original Singapore Food Composition Database contained the levels of 98 nutritive/nonnutritive food components, including folate, and vitamins B_2, B_6 and B_{12}, per 100 g of edible food for each food item included in the FFQ (160). The correlation coefficients for energy intake and selected nutrients from the questionnaire vs. the 24-hour recalls by gender and dialect group were previously reported(160). For example, the correlation coefficients comparing the intake values from questionnaire with those from 24-hour recalls ranged from 0.31 in Cantonese women to 0.53 in Hokkien men for total calories, and 0.50 in Cantonese men to 0.69 in Cantonese women and Hokkien men for calorie-adjusted folate by residual method(166). The

nutrient content information of betaine (167-172), choline (168, 170, 172), and methionine (170, 172) has recently become available and has been added into the Singapore Food Composition Database based on the data published by US Department of Agriculture and the University of Minnesota's Nutrition Coordinating Center Food and Nutrient Database.

Our team has previously reported the correlation coefficients for total calorie intake and selected nutrients (including folate) from the FFQ versus the 24-hour recalls information collected from 858 randomly chosen cohort members for the purpose of validating the FFQ (160). The correlation coefficient ranged from 0.31 to 0.53 for total calories and 0.50 to 0.69 for calorie-adjusted folate by residual method (166) across sex and dialect groups (Cantonese and Hokkien) (160).

2.2.3 Ascertainment of pancreatic cancer cases

Incident pancreatic cancer cases (ICD-Oncology codes, 2nd edition, C25) were identified through record linkage with the databases of the nationwide Singapore Cancer Registry and the Singapore Registry of Births and Deaths. The national cancer registry has been in place since 1968 and has been shown to be comprehensive in recording cancer cases among the entire population (173). As of December 31, 2013, only 57 participants from this cohort were known to be lost to follow-up because of migration out of Singapore (n=30) or for other reasons (n=27). Among those under follow-up, 276 cohort members who were free of cancer at baseline developed pancreatic cancer.

2.2.4 Statistical analysis

Person-years of follow-up time was counted from the date of baseline interview to the diagnosis of pancreatic cancer, lost to follow-up, death of any cause, or December 31, 2013, whichever occurred first. Among the cohort participants, 1,936 had a history of cancer before enrollment, and thus were excluded from the present analysis. Another 459 men and 564 women were excluded due to extreme values of total calorie intake (men: <700 or >3700 kcal/day, women: <600 or >3000 kcal/day). In total, 60,298 subjects including 271 pancreatic cancer cases were included in the analysis.

Nutrient variables were presented as the values adjusted for total calorie intake by using the residual method (166). Cox proportional hazards regression method (174) was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CI) for the associations between one-carbon metabolism-related nutrients and pancreatic cancer risk. Study subjects were grouped into quartiles based on the distribution of nutrient intake in the entire cohort. The nutrients were coded as ordinal values (1, 2, 3, 4) of quartile variables to assess the linear trends of the nutrient-pancreatic cancer association. We did not identify any violation of the proportional hazard assumption.

On the basis of previous analyses in the cohort, we controlled for age at baseline (years), sex, year of baseline interview (1993–1995, 1996–1998), dialect group (Cantonese, Hokkien), and the level of education (no formal schooling, primary school, and secondary school or above) in all multivariable models. We further adjusted for body mass index (BMI) (<18.5, 18.5-21.4, 21.5-24.4, 24.5-27.4, ≥27.5 kg/m^2) (175, 176), smoking status (never smokers, former smokers, current smokers), alcohol drinking (nondrinker, drinker), weekly use of vitamin/mineral supplement (no, yes), self-reported physician diagnosed diabetes (no, yes), any weekly physical

activity (no, yes), and total daily caloric intake (tertiles). Covariates were included in the final multivariable regression models, because including the variable in the base model resulted in change of 10% or more in the hazard ratio for pancreatic cancer and at least one of the one-carbon metabolism-related nutrients, or the variable had been previously reported to be associated with pancreatic cancer in the present study population or other populations.

We further performed stratified analyses by sex. To rule out potential effects of subclinical pancreatic cancer on intake of one-carbon metabolism-related nutrients we performed secondary analyses after excluding pancreatic cancer cases and person-years during the first two years of follow-up post-enrollment. Statistical analyses were conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC). All *P* values were two-sided and considered statistically significant if less than 0.05.

2.3 RESULTS

After an average 16.3 years of follow-up, 271 cohort members (138 males and 133 females) developed pancreatic cancer. The mean age at cancer diagnosis was 72.0 years for males, and 71.6 years for females. The mean time from baseline interview to cancer diagnosis was 10.5 years (range, 3 months to 20.2 years).

Baseline characteristics were evaluated by highest and lowest quartile intake levels of vitamin B_6 and choline, two of the one-carbon metabolism-related nutrients with *a priori* hypothesis for associations with pancreatic cancer risk (**Table 3**). Overall, men (vs. women) were more likely to be ever smokers (57.7% vs. 8.7%) and alcohol drinkers (31.4% vs. 9.0%). Men and women in the highest quartile of vitamin B_6 intake were more likely to have a higher

BMI and to be a never smoker than those in the lowest quartile. Among men, alcohol drinking and diabetes were more prevalent in the highest quartile of vitamin B_6. Men in the highest quartile of choline intake were more likely to be smokers and alcohol drinkers compared to those in the lowest quartile.

Positive correlations were observed between a majority of the one-carbon metabolism-related nutrients (**Table 4**). The strongest correlations were observed between choline and other nutrients, including methionine [Spearman's correlation coefficient (r) = 0.70], vitamin B_2 (r=0.60) and B_{12} (r=0.70). The weakest correlations were observed between methionine and other nutrients, including betaine (r=0.00) and folate (r=0.05). The Spearman's correlation coefficient of vitamin B_6 and choline was 0.50.

Risk of pancreatic cancer in females was 30% lower than that in males (**Table 5**). Compared with never smokers, no association was observed with former smokers, regardless of year since quitting (data not shown). Current smokers who smoked for 30 or more years had an increased pancreatic cancer risk (HR=1.61; 95% CI: 1.19-2.16) that was similar to those who smoked for less than 30 years (HR=1.55; 95% CI: 0.84-2.88), compared with never smokers. Among women, current smokers experienced a statistically significant 93% increased risk of pancreatic cancer compared with never smokers, and alcohol consumption of one or more drinks per week was associated with statistically non-significant 68% increased risk of pancreatic cancer, compared with nondrinkers. There were no significant associations between smoking or drinking and risk of pancreatic cancer in men.

Higher intakes of choline and vitamin B_6 were associated with decreased risk of pancreatic cancer in a dose-dependent manner (**Table 6**). The inverse associations between dietary choline and vitamin B_6 and pancreatic cancer risk remained after excluding the first two

years of follow-up. Comparing the highest versus the lowest quartile, the HRs (95% CIs) for choline and vitamin B_6 were 0.64 (0.45, 0.90) and 0.54 (0.37, 0.78), respectively (both *Ps* for trend $\leq$ 0.02). There was no association for pancreatic cancer risk with the intake of betaine, folate, methionine, vitamins B_2, or B_{12} (**Table 6**). For comparison with a previous reports (102, 158), we evaluated potential joint effects between dietary folate and methionine on pancreatic cancer risk. Although no clear pattern emerged, higher dietary methionine was associated with reduced risk when folate was low (HR=0.64; 95% CI: 0.42-0.99; comparing third to first tertile) (**Table 7**). There was no evidence for joint effects on pancreatic cancer risk with folate and vitamin B_6 or choline, or with vitamin B_6 and choline (all *Ps* for interaction > 0.6).

The association between dietary choline or vitamin B_6 and pancreatic cancer risk was more apparent in men than in women (**Table 6**). However, we did not detect a statistically significant interaction between gender and either choline or vitamin B_6 on pancreatic risk (both *Ps* $\geq$0.15). There were also dose-dependent inverse associations for pancreatic cancer risk only in men for methionine (*P* for trend=0.02) and vitamin B_{12} (*P* for trend=0.047). The gender- nutrient interaction on pancreatic cancer risk was statistically significant for vitamin B_{12} (*P* for interaction=0.01), but not for methionine (*P* for interaction=0.11).

2.4 DISCUSSION

We prospectively evaluated whether one-carbon metabolism-related nutrients were associated with pancreatic cancer risk and found that dietary intake of choline and vitamin B_6 demonstrated inverse, statistically significant trends. Highest quartiles, as compared to the lowest quartiles, of dietary vitamin B_6 and choline were associated with a 48% and 33% decrease in

pancreatic cancer risk, respectively. We reported no association with the other one-carbon metabolism related-nutrients, including betaine, folate, methionine, vitamins B_2 and B_{12}.

Our finding of a dietary vitamin B_6-pancreatic cancer risk association differs from the null results observed in prospective cohorts in Sweden (102) and Finland (103), as well as a large population-based case-control study in the U.S. (158). Possible explanations for the discrepancy include the relatively low intake of vitamin B_6 in our cohort, as well as differences in the major food sources of vitamin B_6 in a Chinese versus Western diet. Less than 15% of our cohort met the U.S. Recommended Daily Allowance of 1.7mg and 1.5mg for men and women, respectively (105). In contrast, 75% of the Swedish cohort and 80% of the U.S. control population had consumed more than 1.7 mg/day from food only (102, 158). The major food sources of vitamin B_6 in our cohort were rice (25%) and fish (16%), compared with meat (29%) and cereals (17%) in Sweden (177), and read-to-eat cereal (13%), poultry (9.0%) and beef (8.7%) in the U.S. (178). The suggestive evidence that red meat intake is associated with an increased risk of pancreatic cancer (179) may partially explain the null results with vitamin B_6 that were observed in Western study populations. It is also possible that once the daily requirement is met there is no additional benefit with higher intake of vitamin B_6 on the risk of pancreatic cancer.

Our dietary vitamin B_6-pancreatic cancer finding supports the statistically significant inverse associations with higher circulating pyridoxal-5'-phosphate (PLP), the bioactive form of vitamin B_6, and pancreatic cancer risk that were reported in two European studies (110, 111), but not the null finding from a pooled analysis of four U.S. cohorts (109). We have previously reported a modest statistically significant correlation with plasma PLP and dietary vitamin B_6 in a healthy subset of our study population ($r = 0.17$, $P=0.0003$) (180). In addition to the lower intake and different major food sources of vitamin B_6 in our study population, perhaps our FFQ

also captured the internal dose more accurately, compared with the studies that reported no association with dietary vitamin B_6 and pancreatic cancer risk.

A protective effect of vitamin B_6 on pancreatic cancer development is biologically plausible given vitamin B_6's role as a cofactor for enzymes involved in the DNA synthesis and methylation pathways of one-carbon metabolism. As a cofactor for serine hydroxymethyltransferase, a diet low in vitamin B_6 results in a decreased production of the methyl donor, methylene-THF (181-183). A decrease in the methylene-THF pool may overload the DNA repair system by increasing uracil incorporation into DNA, and eventually lead to chromosome breaks (183, 184). Global DNA hypomethylation has been linked with genomic instability (185) and tumorigenesis (186, 187). The level of methylation of long interspersed nuclear element-1 (LINE-1) DNA sequences from peripheral lymphocytes is used as a biomarker for genomic DNA methylation status (188), and lower levels are associated with increased risk of some cancers (189). The level of LINE-1 methylation was measured in a healthy subset of our Singapore Chinese cohort (190), and in a secondary analysis, we found that dietary vitamin B_6 had a weak positive correlation with LINE-1 ($r=0.12$, $P=0.007$). In summary, it is biologically plausible that adequate intake of vitamin B_6 may reduce the risk of pancreatic cancer through its beneficial effects on DNA synthesis and methylation status.

Vitamin B_6 may play a role in preventing DNA from oxidative damage. In rats fed a vitamin B_6-deficient diet, decreased activity of pancreatic glutathione reductase, an enzyme that maintains the cellular glutathione level was reported (191). Glutathione is an antioxidant that is required for maintenance of the cellular redox state and detoxification of carcinogens, and low glutathione may impair the antioxidant defense system (152). Therefore, increasing oxidative

stress may represent a mechanistic pathway by which low intake of vitamin B_6 may lead to increased pancreatic cancer risk.

To our knowledge, no epidemiological study has studied the relationship between dietary choline and pancreatic cancer risk. Our observed inverse association between choline intake and pancreatic cancer risk is consistent with the experimental evidence that dietary deficiencies of methyl donors, such as choline, led to aberrant differentiation and function of the exocrine pancreas and contributed to pancreatic carcinogenesis (192). A long-term choline-deficient ethionine (an antagonist of methionine)-supplemented diet in mice with induced chronic pancreatitis resulted in an increase in the expression of key molecules in the pancreatic carcinogenesis process, such as epidermal growth factor receptor (EGFR), K-Ras, and transforming growth factor α (TGF α) (193). Furthermore, a choline-deficient diet was able to shorten the induction period and increase the incidence of carcinogen-induced pancreatic carcinomas in hamsters (100, 194). In summary, our observed inverse association between dietary choline and pancreatic cancer risk is biologically plausible, but it is not clear whether the role of choline is independent of the potential effects of methionine and/or vitamin B_{12}, given that intake of these three nutrients are strongly correlated with each other.

We did not observe statistically significant associations with dietary betaine, folate, methionine, vitamin B_2 or B_{12} for pancreatic cancer risk. Dietary intake of betaine or vitamin B_2 has not been previously evaluated in relation to pancreatic cancer risk. Our finding for no association with folate was consistent with a pooled analysis of 14 prospective cohort studies (65). Our finding for no association with vitamin B_{12} was consistent with results from the only other prospective study to evaluate an association with pancreatic cancer risk (103). Our finding for methionine was similar to two (103, 195), but not all (102) prospective studies that evaluated

methionine-pancreatic cancer risk associations. In our sex-stratified results, a vitamin B_{12}-pancreatic cancer inverse association was only present in men, and vitamin B_{12} was the only nutrient with a statistically significant interaction with sex. Vitamin B_{12} functions as a cofactor for methionine synthase, an enzyme that converts homocysteine to methionine (105). In our data, vitamin B_{12} was strongly correlated with methionine and choline (**Table 4**), making it difficult to tease out the individual effects of these three nutrients on pancreatic cancer risk. The inverse associations with vitamin B_{12}, methionine, and choline intake and pancreatic cancer risk among men in our study suggests that compared to women, men may be more susceptible to low intake of these one-carbon metabolism-related nutrients (196). Our sex-specific findings, however, should be interpreted with caution, as they may be due to chance given the small number of cases available in the stratified analyses.

The strengths of our study include a prospective design, long duration of follow-up, and a comprehensive assessment of one-carbon metabolism-related nutrients. There are also limitations of our study. Due to the nature of an observational study and the one-time assessment of diet, our results may be influenced by misclassification of usual diet during the long follow-up period. However, given the prospective design, the potential for misclassification is unlikely to be different in cases and non-case participants; the non-differential misclassification could bias our results towards the null.

In summary, this prospective cohort study demonstrated statistically significant, inverse associations between dietary vitamin B_6 and choline, and pancreatic cancer risk. These novel findings support the hypotheses that vitamin B_6 and choline are relevant in pancreatic carcinogenesis. Future studies are needed to study the underlying mechanisms how vitamin B_6

and choline, as well as other correlated one-carbon metabolism-related nutrients, may protect against the development of pancreatic cancer.

2.5 TABLES

Table 3. Participant Characteristics According to Intake of Vitamin B_6 (mg) at Baseline, the Singapore Chinese Health Study, 1993-2013

Characteristics	Men				Women			
	Vitamin B_6 intake [a]		Choline intake [a]		Vitamin B_6 intake [a]		Choline intake [a]	
	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
N	8244	6953	8512	6680	6956	8130	15001	15012
Mean age, y	56.8	56.2	56.6	55.9	57.7	54.8	57.7	54.6
Body mass index (kg/m^2), %								
<18.5	7.8	5.5	6.4	7.3	6.8	5.2	5.9	5.8
18.5-21.4	24.7	21.8	23.2	23.1	22.2	22.0	22.1	22.3
21.5-24.4	45.0	42.8	45.4	41.8	47.8	44.3	48.2	43.2
24.5-27.4	16.0	20.7	17.6	18.5	15.0	18.2	15.5	18.5
≥27.5	6.6	9.3	7.4	9.3	8.2	10.2	8.2	10.2
Education, %								
No formal education	12.7	8.1	11.4	9.1	48.8	30.0	48.1	31.1
Primary school	55.0	46.5	53.4	48.7	38.2	40.0	38.0	40.2
Secondary school	32.3	45.4	35.2	42.2	13.0	30.1	13.9	28.7
Smoking status, %								
Never smokers	39.2	44.7	44.6	37.5	88.2	93.9	90.3	91.6
Former smokers	21.1	22.2	22.3	20.2	3.2	2.1	3.0	2.4
Current smokers	39.7	33.1	33.1	42.3	8.6	4.0	6.8	6.1
Alcohol drinking, %								
Nondrinkers	74.5	61.0	74.6	57.9	90.6	90.1	92.1	88.4
<7 drinks/week	20.5	23.1	21.1	24.2	7.7	86	6.7	10.1
≥7 drinks/week	5.0	15.9	4.4	17.9	1.7	1.4	1.2	1.6
Weekly vitamin / minerals use (% Yes)	3.9	6.5	4.1	5.9	5.7	10.5	5.6	9.5
Weekly physical activity (% Yes)	42.1	46.9	44.6	41.7	21.3	30.1	22.6	27.5
Diabetes (% Yes)	6.9	9.5	6.8	10.3	8.4	8.2	8.0	10.1

Table 3 continued

Characteristics	Men				Women			
	Vitamin B_6 intake [a]		Choline intake [a]		Vitamin B_6 intake [a]		Choline intake [a]	
	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
Mean calorie intake, kcal/day	1879.8	1809.2	1878.6	1827.3	1540.1	1486.2	1415.8	1645.8
Mean nutrient intake[b]								
Betaine, mg/day	63.4	81.4	62.8	79.3	71.1	76.6	69.5	74.9
Choline, mg/day	188.7	259.8	160.4	306.0	199.7	260.8	174.3	292.4
Folate, µg/day	119.2	185.3	127.8	169.2	129.6	194.2	138.0	178.8
Methionine, mg/day	1171.2	1400.6	1098.0	1540.6	1208.5	1471.7	1129.8	1597.5
Vitamin B_2, mg/day	0.8	1.0	0.7	1.1	0.9	1.1	0.8	1.1
Vitamin B_6, mg/day	0.8	1.4	1.0	1.2	0.9	1.4	1.0	1.2
Vitamin B_{12}, µg/day	1.8	2.6	1.5	3.1	2.0	2.7	1.6	3.1

Abbreviations: Q1: 1st quartile; Q4, 4th quartile.

[a] Nutrient intake was adjusted for daily total calorie intake by residual method. Quartiles of vitamin B_6 and choline were based on the distribution among the entire cohort.

[b] Nutrient intake was adjusted for daily total calorie intake by residual method.

Table 4. Spearman correlation coefficients between energy-adjusted dietary intake of one-carbon metabolism-related nutrients[a], the Singapore Chinese Health Study, 1993-2013[b]

	Betaine	Choline	Folate	Methionine	Vitamin B_2	Vitamin B_6	Vitamin B_{12}
Betaine		0.14	0.35	0.00[c]	0.37	0.12	0.06
Choline			0.36	0.70	0.60	0.50	0.70
Folate				0.05	0.51	0.54	0.14
Methionine					0.38	0.38	0.76
Vitamin B_2						0.37	0.52
Vitamin B_6							0.34

[a]Nutrient intake was adjusted for daily total calorie intake by residual method.
[b]All 2-sided $P < 0.0001$; [c]2-sided $P = 0.92$

Table 5. Associations between potential risk factors and pancreatic cancer risk overall, and stratified by sex, the Singapore Chinese Health Study, 1993-2013

Characteristics	Total subjects		Men		Women	
	Cases, N	HR (95%CI)[a]	Cases, N	HR (95%CI)[a]	Cases, N	HR (95%CI)[a]
Age	271	1.09 (1.08-1.11)	138	1.10 (1.07-1.12)	133	1.09 (1.06-1.12)
Female vs. Male	271	0.70 (0.55-0.89)	138	---	133	---
Body mass index, kg/m^2						
<18.5	22	1.44 (0.91-2.27)	13	1.53 (0.84-2.79)	9	1.33 (0.66-2.69)
18.5-21.4	62	1.18 (0.86-1.60)	37	1.27 (0.84-1.92)	25	1.06 (0.66-1.70)
21.5-24.4	114	1.00 (ref)	57	1.00 (ref)	57	1.00 (ref)
24.5-27.4	55	1.33 (0.97-1.84)	26	1.20 (0.75-1.90)	29	1.48 (0.95-2.32)
≥27.5	18	0.93 (0.56-1.52)	5	0.58 (0.23-1.45)	13	1.21 (0.66-2.21)
Education						
No formal education	82	1.00 (ref)	14	1.00 (ref)	68	1.00 (ref)
Primary school	121	1.07 (0.78-1.45)	75	1.30 (0.73-2.32)	46	1.00 (0.68-1.49)
Secondary school	68	1.16 (0.81-1.68)	49	1.45 (0.79-2.67)	19	1.02 (0.60-1.76)
Smoking						
Never smokers	166	1.00 (ref)	54	1.00 (ref)	112	1.00 (ref)
Former smokers	34	0.96 (0.64-1.45)	31	0.92 (0.59-1.44)	3	0.76 (0.24-2.41)
Current smokers	71	1.40 (1.02-1.92)	53	1.24 (0.84-1.81)	18	1.93 (1.17-3.20)
Alcohol drinking						
Nondrinkers	222	1.00 (ref)	105	1.00 (ref)	117	1.00 (ref)
< 7 drinks/week	39	1.00 (0.71-1.42)	24	0.77 (0.49-1.20)	15	1.68 (0.98-2.89)[b]
≥ 7 drinks/week	10	0.80 (0.42-1.52)	9	0.79 (0.40-1.56)	1	--
Diabetes						
No	241	1.00 (ref)	122	1.00 (ref)	119	1.00 (ref)
Yes	30	1.30 (0.88-1.90)	16	1.49 (0.88-2.52)	14	1.19 (0.64-1.95)
Weekly vitamin/mineral supplement use						
No	256	1.00 (ref)	132	1.00 (ref)	124	1.00 (ref)
Yes	15	0.89 (0.53-1.51)	6	0.80 (0.35-1.82)	9	0.99 (0.50-1.94)

[a] HR and 95% CI were adjusted for age (years), sex, father's dialect group (Cantonese, Hokkien), and year of interview (1993–1995, 1996–1998).
[b] HR and 95% CI were calculated for drinkers vs. nondrinkers. Only one case was in women ≥ 7 drinks/week, and thus < 7 drinks/week was combined with ≥ 7 drinks/week.

Table 6. Multivariate analysis of one-carbon metabolism dietary factors and pancreatic cancer incidence by sex, Singapore Chinese Health Study 1993-2013

Nutrients	Median intake[a]	All		Men		Women	
		Cases, N	HR (95%CI)[b]	Cases, N	HR (95%CI)[b]	Cases, N	HR (95%CI)[b]
Betaine, mg/day							
Q1	41.56	68	1.00 (ref)	43	1.00 (ref)	25	1.00 (ref)
Q2	59.43	75	1.12 (0.81-1.57)	40	1.21 (0.78-1.87)	35	1.10 (0.65-1.83)
Q3	70.84	71	1.03 (0.73-1.44)	29	0.82 (0.51-1.33)	42	1.30 (0.79-2.14)
Q4	105.62	57	0.80 (0.56-1.14)	26	0.66 (0.40-1.08)	31	1.05 (0.62-1.79)
P-trend			0.19		0.051		0.71
Choline, mg/day							
Q1	176.30	97	1.00 (ref)	62	1.00 (ref)	35	1.00 (ref)
Q2	217.25	54	0.57 (0.40-0.80)	22	0.48 (0.29-0.78)	32	0.70 (0.43-1.13)
Q3	245.27	64	0.72 (0.52-1.00)	29	0.71 (0.45-1.11)	35	0.79 (0.49-1.27)
Q4	286.79	56	0.67 (0.48-0.93)	25	0.55 (0.34-0.88)	31	0.86 (0.53-1.41)
P-trend			0.04		0.02		0.69
Folate, μg/day							
Q1	108.24	74	1.00 (ref)	45	1.00 (ref)	29	1.00 (ref)
Q2	137.56	66	0.94 (0.67-1.32)	33	1.00 (0.63-1.58)	33	0.92 (0.55-1.52)
Q3	162.90	74	1.12 (0.81-1.56)	30	0.94 (0.58-1.50)	44	1.35 (0.83-2.17)
Q4	207.21	57	0.89 (0.63-1.28)	30	0.90 (0.56-1.44)	27	0.94 (0.54-1.61)
P-trend			0.82		0.62		0.73
Methionine, mg/day							
Q1	1073.17	72	1.00 (ref)	47	1.00 (ref)	35	1.00 (ref)
Q2	1268.95	88	1.26 (0.92-1.73)	48	1.35 (0.90-2.04)	40	1.17 (0.71-1.92)
Q3	1414.90	58	0.88 (0.62-1.25)	20	0.62 (0.37-1.05)	38	1.14 (0.69-1.89)
Q4	1625.25	53	0.82 (0.57-1.17)	23	0.67 (0.41-1.10)	30	1.02 (0.60-1.72)
P-trend			0.09		0.02		0.98

Table 6 continued

Nutrients	Median intake[a]	All		Men		Women	
		Cases, N	HR (95%CI)[b]	Cases, N	HR (95%CI)[b]	Cases, N	HR (95%CI)[b]
Vitamin B_2, mg/day							
Q1	0.71	71	1.00 (ref)	48	1.00 (ref)	23	1.00 (ref)
Q2	0.86	70	1.03 (0.74-1.44)	34	0.96 (0.61-1.51)	36	1.20 (0.71-2.04)
Q3	0.98	61	0.92 (0.65-1.30)	31	0.86 (0.54-1.36)	30	1.08 (0.63-1.87)
Q4	1.20	69	1.01 (0.72-1.42)	25	0.68 (0.42-1.11)	44	1.55 (0.93-2.59)
P-trend			0.88		0.12		0.13
Vitamin B_6, mg/day							
Q1	0.88	95	1.00 (ref)	62	1.00 (ref)	33	1.00 (ref)
Q2	1.02	64	0.71 (0.51-0.98)	22	0.48 (0.29-0.80)	42	1.03 (0.64-1.64)
Q3	1.13	68	0.80 (0.58-1.11)	31	0.73 (0.47-1.14)	37	0.98 (0.60-1.58)
Q4	1.33	44	0.52 (0.36-0.74)	23	0.45 (0.28-0.74)	21	0.66 (0.38-1.15)
P-trend			0.001		0.004		0.16
Vitamin B_{12}, μg/day							
Q1	1.43	74	1.00 (ref)	54	1.00 (ref)	20	1.00 (ref)
Q2	2.05	69	0.97 (0.69-1.35)	29	0.71 (0.45-1.12)	40	1.57 (0.91-2.70)
Q3	2.52	68	0.99 (0.70-1.38)	34	0.91 (0.59-1.41)	34	1.31 (0.75-2.28)
Q4	3.26	60	0.88 (0.62-1.24)	21	0.54 (0.33-0.90)	39	1.60 (0.93-2.74)
P-trend			0.51		0.047		0.21

[a] Nutrient intake levels were adjusted for daily total calorie intake using residual method.

[b] Adjusted for age (continuous, year), sex (male, female), year of interview (1993–1995, 1996–1998), dialect group (Cantonese, Hokkien), education (no formal education, primary school, and secondary school or higher), BMI (<18.5, 18.5-21.4, 21.5-24.4, 24.5-27.4, ≥27.5 m/kg^2), smoking status (never, former, current), diabetes (no, yes), alcohol drinking (no, yes), and weekly vitamin use (no, yes). Stratified analyses were not adjusted by sex. The cutoff values for one-carbon metabolism-related nutrients were the same for men and women.

Table 7. Joint effects between dietary folate and methionine on pancreatic cancer risk, the Singapore Chinese Health Study, 1993-2013

	Folate			
	<median, 149.4 ug/day		≥median, 149.4 ug/day	
	Cases, n	OR (95% CI)	Cases, n	OR (95% CI)
Methionine (median, mg/day)				
1st tertile (1117.7)	59	1.00 (ref)	39	0.79 (0.52-1.19)
2nd tertile (1340.7)	49	0.89 (0.61-1.31)	54	1.12 (0.76-1.63)
3rd tertile (1575.8)	32	0.64 (0.42-0.99)	38	0.78 (0.52-1.18)
P for interaction = 0.19				

[a] Nutrient intake levels were adjusted for daily total calorie intake using residual method.
[b] Adjusted for age, sex, year of interview, dialect group, education, body mass index, smoking status, diabetes, alcohol drinking, and weekly vitamin use.

3.0 SERUM B_6 VITAMERS (PYRIDOXAL 5'-PHOSPHATE, PYRIDOXAL, AND 4-PYRIDOXIC ACID) AND PANCREATIC CANCER RISK: TWO NESTED CASE-CONTROL STUDIES IN ASIAN POPULATIONS

Background: Vitamin B_6 is an important enzymatic cofactor in pathways relevant for the development of pancreatic cancer. In order to evaluate vitamin B_6 as a preventive factor for pancreatic cancer, a biomarker approach is needed to overcome the limitations inherent in self-reported dietary information. **Methods:** To determine whether levels of serum B_6 vitamers, including pyridoxal 5'-phosphate (PLP), pyridoxal (PL), 4-pyridoxic acid (PA), and the PA:(PLP+PL) ratio (PAr) were associated with risk of pancreatic cancer, two nested case-control studies of 187 incident pancreatic cancer cases and 258 individually matched controls were conducted within two prospective cohorts of 81 501 participants in Shanghai, China, and Singapore. PLP, PL, and PA were quantified in pre-diagnostic serum samples. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression with adjustment for potential confounders. **Results:** The median (5^{th}-95^{th} percentiles) concentrations of serum PLP among control subjects of the Shanghai and Singapore cohorts were 25.7 (10.0-91.7) nmol/L and 58.1 (20.8-563.0) nmol/L, respectively. In pooled analyses, high serum PLP was associated with a reduced risk of pancreatic cancer (P for trend = 0.048); the adjusted odds ratio for the highest category of PLP (>52.4 nmol/L) was 0.46 (95% confidence interval: 0.23, 0.92) compared to vitamin B_6 deficiency (<20 nmol/L). No associations were found for serum PL, PA, or PAr with pancreatic cancer risk. **Conclusions:** Higher concentrations of PLP may

protect against the development of pancreatic cancer. The protective effect may be more apparent in populations with low concentrations of circulating vitamin B_6.

3.1 INTRODUCTION

Pancreatic cancer is the seventh leading cause of cancer–related death in the world, with an estimated 331 000 deaths due to pancreatic cancer in 2012 (197). Cigarette smoking and excess body fatness are of the few established modifiable risk factors for pancreatic cancer (33, 46), and studies are needed to identify novel targets of primary prevention for pancreatic cancer. Consumption of fruits and vegetables has been associated with reduced risk of pancreatic cancer in some epidemiological studies (144, 198). However, the associations between specific nutrients and pancreatic cancer risk have not been well studied.

Vitamin B_6 is present in a wide variety of foods such as beef liver, tuna, and bananas (199). We recently reported an inverse association between dietary intake of vitamin B_6 and risk of pancreatic cancer in a prospective cohort of Chinese in Singapore; there was a 48% reduction in risk of developing pancreatic cancer associated with the highest (>1.21 mg/day) versus lowest (<0.96 mg/day) quartile of vitamin B_6 intake (200). However, similar studies in populations with higher intake (i.e., Europe and US) did not observe inverse associations [i.e., highest (≥2.22-2.81 mg/day) versus lowest quartiles (<1.77-2.09 mg/day)] (102, 103, 158). It is possible that the etiologically relevant range of intake was not captured in the European and US populations, or the discrepancies could be due to the inherent limitation of measurement error associated with assessing dietary vitamin B_6 intake from food frequency questionnaires. A biomarker approach for vitamin B_6 and its related metabolites in bodily fluid would overcome the limitation of

relying on self-reported diet, and it would provide insights on the potential role of the various B_6 vitamers in the development of pancreatic cancer.

Pyridoxal 5'-phosphate (PLP), the metabolically active form of vitamin B_6, is a coenzyme in the synthesis of nucleic acids, amino acids, and cellular antioxidants (152). PLP accounts most of the total vitamin B_6 in the circulation and is commonly used as a primary measure of whole-body vitamin B_6 status (201). Besides PLP, other major forms of vitamin B_6 in the circulation in humans include pyridoxal (PL) and 4-pyridoxic acid (PA) (202). The ratio of PA to the sum of PLP and PL (PAr) is speculated as a marker of increased vitamin B_6 catabolism during inflammation (115). Recently, PAr has been shown to be positively associated with several inflammatory markers (115), thus suggesting PAr may be a biomarker for pancreatic cancer risk (203). Given the inconsistent results on the relationship between circulating PLP concentration and pancreatic cancer risk (109-111), we conducted a comprehensive assessment of the individual B_6 vitamer levels, as well as PAr in relation to pancreatic cancer risk in two prospective cohorts of Asians in order to clarify the potential role of vitamin B_6 in pancreatic cancer development.

3.2 METHODS

3.2.1 Study population

The design of the Shanghai Cohort Study has been described in detail elsewhere (204). Briefly, 18,244 men aged 45 to 64 years in Shanghai, China were enrolled between 1986 and 1989. At the time of recruitment, all participants were interviewed in person by a trained nurse

using a structured questionnaire that asked for information on demographics, height, weight, use of tobacco and alcohol, and medical history. In addition, each study participant provided a non-fasting blood samples and a spot urine sample following the interview. All collected biospecimens were kept on ice (at around 4 °C) before they were processed and aliquots of serum and urine specimens have been stored at -80 °C until laboratory analysis.

The design of the Singapore Chinese Health Study has been described in detail elsewhere (159). Briefly, 63,257 Chinese men and women aged 45-74 years in Singapore were enrolled between 1993 and 1998. At the time of recruitment, all participants were interviewed in person using a structured questionnaire including sections of background information, occupational exposure, physical activity, and family history of cancer and provided information on height, weight, use of tobacco and alcohol, dietary supplemental use, and medical history. Information on habitual diet was collected using a validated 165-item food frequency questionnaire (160). Daily intake of nutrients including vitamin B_6 was calculated using the nutrient content information from the Singapore Food Composition Database (160). Non-fasting blood samples and spot urine samples were collected from a 3% random sample of cohort members between 1994 and 1999, and extended to all surviving cohort members between 2000 and 2005. By April 2005, blood and/or urine specimens were collected from 32,543 participants, representing a consent rate of 60%. Serum and urine specimens were kept in insulated boxes with ice (4 °C) until processing, and stored at -80 °C. For Singapore subjects, blood sample was collected on average 6.5 (range, 1.2-11.0) years after the baseline interview. Follow-up I interview (N=52,322) was administered during 1999-2003 and the consent rate reached over 90% among surviving cohort members. Since the status of smoking and diabetes may change over time, the information on smoking and diabetes were derived mainly from follow-up I interview (98%)

supplemented by baseline interview (2%). A validation study of the incident diabetes cases in the Singapore cohort observed that 99% of individuals who reported a history of diabetes were considered valid cases (205). Another study analyzed percentage of hemoglobin A1c (HbA1c) (glycated hemoglobin) among individuals who reported no history of diabetes at baseline and follow-up interview and observed 94.4% of those individuals were below the HbA1c threshold for diabetes (206). Other demographic and lifestyle factors used were derived from the baseline interview only.

Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the Shanghai Cancer Institute, the National University of Singapore, and the University of Pittsburgh.

3.2.2 Case ascertainment and control selection

In the Shanghai cohort, all surviving cohort participants were re-contacted annually and interviewed in-person to update the information on selected lifestyle factors and medical history. As of the most recent follow-up in 2015, 3.7% of original cohort participants were lost to the follow-up interview and 3.3% declined the continued follow-up interview. The incident cancer cases and deaths among cohort participants were identified through annual re-contacts of surviving study participants or next-of-the-kin for deceased participants, and through record linkage analyses with the databases of the population-based Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Office. The diagnosis of all incident cancer cases was confirmed via review of medical records. As of December 31st 2009, the cut-off date for the present study, 129 incident cases of pancreatic cancer [International Classification of Disease (ICD)-9 code, 157] were identified among participants of the Shanghai cohort.

In the Singapore cohort, less than 1% of original cohort members were lost to follow-up due to their migration out of Singapore. The incident cancer cases and deaths among cohort members of the Singapore cohort were identified through routine record linkage with databases of the Singapore National Birth and Death Registry and National Cancer Registry (173). As of December 31st, 2013, 58 incident pancreatic cancer cases (ICD-Oncology code, C25) were identified among participants of the Singapore cohort who had available serum samples.

For each case, two control subjects were randomly selected among all eligible participants who were free of cancer at the time of cancer diagnosis of the index case within the same cohort. To be consistent with the matching criteria used in previous nested case-control studies in the Shanghai cohort, controls were matched to the index case on date of birth (±2 years), date of biospecimen collection (±1 month), and neighborhood of residence at time of enrollment (207). In the Singapore cohort, cases and controls were matched on age at baseline interview (± 3 years), date of baseline interview (± 2 years), gender, dialect group (Cantonese, Hokkien), and date of biospecimen collection (±6 months).

3.2.3 Assessment of serum biomarkers

For each subject, 60 µL serum was pulled from the biorepository. Serum PLP, PL, PA, and creatinine were measured by liquid chromatography-tandem mass spectrometers (LC-MS/MS) using the methods described previously (208). All biochemical analyses were performed at Bevital A/S (www.bevital.no) at Bergen, Norway. Serum specimens of cases and their matched controls were processed, aliquoted, shipped in insulated boxes with dry ice, and assayed together in the same batch. Laboratory technicians were blinded about case-control status of the test samples. For quality control purposes, 14 duplicated samples (2% of testing

samples) from a pooled serum sample collected from potential study subjects for the Shanghai Cohort Study but later determined ineligible were included in 7 batches (2 duplicated samples per batch). The within-batch coefficients of variation (CVs) for PLP, PL, PA, and creatinine were 3.3%, 7.5%, 6.0%, and 3.3%, respectively. The corresponding between-batch CVs were 7.9%, 8.1%, 7.5%, and 4.0%.

3.2.4 Statistical analysis

PAr was calculated by dividing serum concentrations of PA by the sum of PL and PLP. We logarithmically transformed original values of PLP, PL, PA and PAr to normalize their skewed distributions towards high values. Pairwise correlations between biomarkers of PLP, PL, PA, and PAr were evaluated using Spearman correlation coefficients. The differences in concentrations of PLP, PL, PA, and the value of PAr between different categories of baseline demographic characteristics and lifestyle factors were evaluated using Analysis of Covariance (ANCOVA).

Conditional logistic regression (209) was used to calculate odds ratios (ORs) and their 95% confidence intervals (CIs) of pancreatic cancer associated with higher categories of PLP, PL, PA, and PAr, comparing with the lowest category. In the primary analysis of both cohorts combined, we used <20 nmol/L PLP as the lowest (i.e., reference) category for OR because it has been suggested as a cutpoint for vitamin B_6 deficiency (210), and divided the remaining total subjects into equal tertiles based on the distribution of PLP among controls of both cohorts. For PL, PA, and PAr, study subjects were divided into quartiles based on the distribution of individual biomarkers among total controls. In cohort-specific analysis, quartiles of PLP, PL, PA and PAr were derived from their distributions among controls within each cohort. The

heterogeneity in the biomarker-pancreatic cancer risk association between two cohorts was assessed using the method described previously (211). Ordinal values (e.g., 1, 2, 3, and 4) for each of biomarkers were used for testing linear trend in the biomarker-pancreatic cancer risk association.

The multivariable logistic regression models included following reported risk factors for pancreatic cancer as potential confounders: body mass index (BMI) (<18.5, 18.5-<23, ≥23), level of education (no formal schooling, primary school, secondary school and above), smoking status (never smokers, former smokers, current smokers), alcohol consumption (number of drinks per day), and history of physician-diagnosed diabetes (no, yes). Given the impact of renal clearance on PA (202), we further adjusted for estimated glomerular filtration rate (eGFR) (212) in the analysis for the association between PA, PAr, and pancreatic cancer risk.

To minimize the potential residual confounding of diabetes, we conducted a sensitivity analysis by excluding subjects who reported a history of diabetes. In addition, to reduce the potential effect of disease progression on diminishing circulating B_6 vitamers, we conducted separate analysis after excluding cases diagnosed within 2 years after blood draw and their matched controls.

Statistical analyses were carried out using SAS software version 9.3 (SAS Institute, Cary, NC) All *P* values reported are two-sided, and those that were <0.05 were considered to be statistically significant.

3.3 RESULTS

The mean age at pancreatic cancer diagnosis was 69.0 and 71.7 years in the Shanghai and Singapore cohorts, respectively. The average (range) time between blood draw and cancer diagnosis was 12.5 years (3 months to 23.2 years) for cases of the Shanghai cohort, and 6.8 (5 months to 13.0 years) for cases of the Singapore cohort. Patients who developed pancreatic cancer were more likely to smoke cigarettes at baseline in the Shanghai cohort, whereas the distributions of smoking status between cases and controls in the Singapore cohort were comparable (**Table 8**). Overall circulating mean levels of PLP, PL and PA were 20% to 56% lower in controls of the Shanghai cohort than those of the Singapore cohort. Compared with controls, patients who developed pancreatic cancer had lower serum levels of PLP and PL at baseline in the Shanghai cohort and similar levels in the Singapore cohort. No difference in PA and PAr between cases and controls was seen in both cohorts. Serum concentrations of PLP, PL, and PA were highly correlated with each other in the study population (**Table 9**).

Current smokers showed the lowest concentrations of serum PLP, PL, and PA among controls of both cohorts whereas smoking status was not associated with PAr (**Table 10**). Alcohol intake was inversely associated with PAr in the Shanghai study controls. In Singapore cohort, controls who reported use of multivitamins showed a markedly increase in concentrations of PLP, PL, and PA, and PAr compared to non-users. PAr was higher in diabetic patients than non-diabetics in both cohorts. Lower renal function (i.e., low eGFR) was associated with higher levels of PL, PA, and PAr in both cohorts.

High levels of PLP were associated with reduced risk of pancreatic cancer (**Table 11**). Compared with PLP < 20 nmol/L, subjects with PLP >52.4 nmol/L at baseline had a 59% reduced risk of developing pancreatic cancer. Adjustment for level of education, BMI, cigarette

smoking, alcohol intake, and history of diabetes slightly attenuated the association with PLP (**Table 11**). Circulating PL and PA level were inversely associated with pancreatic cancer risk (both P trend values ≥ 0.06), and these associations were further attenuated with the adjustment for potential confounders. No association between PAr and pancreatic cancer risk was observed. In cohort-specific analysis, we used quartile levels of all B_6 vitamers for the risk association analysis because there were very few subjects with PLP <20 nmol/L in the Singapore cohort (**Table 12**). The inverse association between PLP and risk of pancreatic cancer was stronger in the Shanghai cohort (P=0.01) compared with the Singapore cohort (P=0.58) (**Table 13**). There was no evidence for associations between other biomarkers of vitamin B_6 and pancreatic cancer in either cohort.

Excluding cases and controls with a history of diabetes (7 cases and 14 controls), the inverse association between serum PLP and pancreatic cancer risk remained; the multivariable-adjusted ORs (95% CIs) for the 2^{nd}, 3^{rd}, and 4^{th} quartile of PLP were 0.70 (0.41-1.20), 0.72 (0.4-1.29), and 0.45 (0.22-0.93), respectively, compared with <20 nmol/L (P for trend = 0.048). Excluding cases (n=13) whose blood samples were collected within 2 years prior to pancreatic cancer diagnosis and their matched controls (n=26) did not appreciably change the association with PLP. The multivariable-adjusted OR of pancreatic cancer for PLP >52.4 nmol/L relative to PLP <20 nmol/L was 0.45 (0.22-0.94) (P trend=0.056). No association of PL, PA, or PAr with pancreatic cancer risk was found.

3.4 DISCUSSION

The present study demonstrated that higher concentrations of PLP in serum collected many years before cancer diagnosis were associated with reduced risk of developing pancreatic cancer in two prospective cohorts of Chinese populations. Compared with vitamin B_6-deficient individuals, participants with PLP at the highest quartile (>52.4 nmol/L) had a 54% reduced risk of pancreatic cancer. These results supported an inverse association between dietary intake of vitamin B_6 and pancreatic cancer risk that we reported previously in the Singapore cohort (200), and suggest that vitamin B_6 may play a protective role in the development of pancreatic cancer. The present study did not demonstrate an association for serum levels of PL, PA, and PAr with risk of pancreatic cancer.

In the cohort-specific analysis, the inverse association between PLP and pancreatic cancer risk was found in the Shanghai cohort but not in the Singapore cohort. The lack of association in the Singapore cohort was primarily due to the small sample size and relatively higher level of PLP. Overall only 5.4% of the Singapore study controls who did not report multivitamin use showed PLP <20 nmol/L, thus cohort-specific quartile cut-off values were used in the analysis, resulting in a median concentration of 31.7 nmol/L PLP of the lowest quartile. This is not surprising for a relatively weak inverse association between PLP and pancreatic cancer risk in the Singapore cohort study in which a high level of PLP as a reference group was observed. It is interesting to note that the overall incidence rate of pancreatic cancer is approximately 38% higher in Singapore than in Shanghai, China (4.86 versus 6.78 per 100 000 men) based on the GLOBOCAN 2012 estimates (197). It is possible that the observed association between serum PLP and risk of pancreatic cancer was underestimated given the

higher incidence rate of pancreatic cancer in Singapore, with relatively higher concentrations of serum PLP, compared with Shanghai populations.

The inverse association between serum PLP and pancreatic cancer in our study is consistent with some previous studies but not others. There were three previous studies that evaluated associations between circulating PLP and risk of pancreatic cancer. The first was a nested case-control study among current smokers that included 126 cases of pancreatic cancer and 247 matched control subjects within the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study in Finland (**Table 14**). The ATBC study reported an inverse association between serum PLP, determined by the enzymatic method (i.e., tyrosine decarboxylase apoenzyme method) (213), and pancreatic cancer risk; OR was 0.48 for the highest (>39.46 nmol/L) versus lowest tertile (<26.34 nmol/L) of PLP (*P* for trend = 0.02) (111). The second study was also a nested case-control study of pancreatic cancer pooled from four U.S. cohorts including the Nurses' Health Study, the Health Professionals Follow-up Study, the Physicians' Health Study, and the Women's Health Initiative involving 208 cases and 623 controls (109). That study reported a slightly reduced risk of pancreatic cancer associated with highest quartile of PLP (OR = 0.87, 95% CI = 0.55-1.37). It is worth noting that a radioenzymatic assay was used to quantify plasma PLP that yielded an average of 12.7 nmol/L PLP (109), which was well below 20 nmol/L as the cut-off value for vitamin B_6 deficiency (210). This level was 65% to 80% lower than the median plasma PLP (39.0 to 60.2 nmol/L) that was recently measured using the LC-MS/MS method in plasma samples from these same 4 cohorts, which were part of the Lung Cancer Cohort Consortium project (per communication, Øivind Midttun, 2015). The LC-MS/MS quantification of these samples was conducted by the same laboratory as our study samples in the present study. The third nested case-control study was conducted within the European

Prospective Investigation into Cancer and Nutrition cohort and also found an, inverse association between plasma PLP, determined by the same LC-MS/MS method, and pancreatic cancer risk in women (OR=0.4, 95% CI=0.2–0.8 for 5^{th} versus 1^{st} quintile of PLP) but not in men (OR = 1.1, 95% CI=0.6-2.3) (110). The inconsistent results from the previous studies could be attributed to different levels of exposure to vitamin B_6 across different study populations and different methods used in PLP quantification.

Vitamin B_6 may play a role in protecting DNA against oxidative damage and subsequent mutations and therefore reduce the potential to develop cancer. As a cofactor for cystathionine β-synthase and cystathionine γ-lyase, PLP is involved in the production of the important cellular antioxidant glutathione. In addition to its cofactor role, vitamin B_6 may serve act as a scavenger of reactive oxidative species (214). In men, serum vitamin B_6 was inversely associated with urinary 8-hydroxydeoxyguanosine, a marker of DNA oxidative damage (215). It has recently been shown that PLP deficiency resulted in formation of advanced glycation end products (AGEs), a major contributor of cellular oxidative stress, and subsequent chromosome aberrations in HeLa cells (216). Interestingly, higher serum concentrations of soluble receptors for AGEs that neutralizes and blocks the effect of AGEs were associated with reduced risk of multiple cancers including pancreatic cancer (217, 218). Future studies are warranted to study the biological pathways underlying the potential protective effect of PLP against pancreatic cancer development.

PLP is the primary form of circulating vitamin B_6 and accounts for 70-90% of the total circulating B_6 vitamers (i.e., sum of PLP, PL and PA) (219). PL is the transport form of PLP across cellular membranes, while PA is the vitamin B_6 catabolite excreted through urine (202). Our study evaluated serum concentrations of PL and PA and pancreatic cancer risk and did not

find any associations. Therefore, PLP may be more relevant in pancreatic carcinogenesis compared with PL and PA. No previous study has evaluated the association between PAr and pancreatic cancer risk. In the current study, we found no association between PAr, a marker of vitamin B_6 catabolism (115), and pancreatic cancer risk. In summary, overall vitamin B_6 status (e.g., PLP) rather than vitamin B_6 catabolism (e.g., PAr) may be more relevant in pancreatic carcinogenesis.

The strengths of our study are the prospective study design and using a LC-MS/MS based method with high accuracy and precision to quantify the B_6 vitamers. In addition, compared with the higher levels of PLP in the Singapore cohort and the US and European populations, the Shanghai cohort provided a unique study population to examine the PLP-pancreatic cancer association at the lower end of the exposure spectrum. Moreover, the relatively long time interval between blood collection and pancreatic cancer diagnosis (on average 12.5 years in the Shanghai cases and 6.8 years in the Singapore cases) would diminish the potential impact of disease progression or subclinical symptoms on the circulating PLP concentration. Our study was limited by having a small sample size, especially in the Singapore cohort. Studies with a larger sample size in other study populations are warranted to validate our findings.

In conclusion, our study suggested that sufficient PLP in serum was associated with a 54% reduced risk of pancreatic cancer in a pooled analysis of two prospective cohorts of Asians. These results suggest that a diet high in vitamin B_6 may be protective against the development of pancreatic cancer, especially in populations with relatively low levels of *in vivo* vitamin B_6. Although vitamin B_6 deficiency in developed countries is rare, certain groups are at higher risk of marginal vitamin B_6 status including the elderly, pregnant women, individuals taking certain drugs, and chronic alcohol abusers (202). In addition to replication in other observational studies,

further studies are needed to investigate the potential mechanisms by which PLP exerts its role against the development of pancreatic cancer.

3.5 TABLES

Table 8. Baseline demographic characteristics and lifestyle factors of pancreatic cancer cases and control subjects, The Shanghai Cohort Study (Shanghai) and The Singapore Chinese Health Study (Singapore)

Baseline Characteristics	Shanghai cohort			Singapore cohort		
	Controls	Cases	P[a]	Controls	Cases	P[a]
N	258	129		104	58	
Age at interview, mean (SD), years	56.4 (5.5)	56.5 (5.5)	0.74	57.1 (7.2)	57.9 (7.5)	0.51
Age at blood draw, mean (SD), years	56.4 (5.5)	56.5 (5.5)	0.74	64.0 (7.1)	64.9 (7.6)	0.47
BMI, mean (SD), kg/m^2	21.9 (2.8)	22.5 (3.0)	0.08	23.1 (3.2)	23.2 (3.6)	0.79
Female (%)	0	0		39.4	39.7	0.98
Education level (%)			0.36			0.42
No formal schooling	5.0	2.3		20.2	12.1	
Primary school	28.7	26.4		43.3	48.3	
Secondary school or above	66.3	71.3		36.5	39.7	
Smoking status (%)			0.003			0.87
Never	43.8	27.1		60.6	58.6	
Former	6.2	4.7		22.1	20.7	
Current	50.0	68.2		17.3	20.7	
Alcohol intake, drinks/week (%)			0.74			0.61
0	56.6	54.3		82.7	87.9	
<7	11.2	14.0		10.6	8.6	
≥7	32.2	31.8		6.7	3.5	
Diabetes (%)			0.52			0.88
No	98.5	99.2		90.4	89.7	
Yes	1.55	0.78		9.6	10.3	
Weekly multivitamin use (%)			---			0.69
No	---	---		89.4	91.4	
Yes	---	---		10.6	8.62	

Table 8 continued

Baseline Characteristics	Shanghai cohort			Singapore cohort		
	Controls	Cases	P[a]	Controls	Cases	P[a]
Serum biomarker concentrations (median, 5th-95th)						
PLP (nmol/L)	25.7 (10.0-91.7)	21.7 (8.9-60.0)	0.01	58.1 (20.8-563.0)	50.6 (23.8-465.0)	0.29
PL (nmol/L)	15.0 (15.0-55.0)	14.0 (6.8-34.8)	0.03	20.0 (8.4-2680.0)	21.1 (8.2-3960.0)	0.84
PA (nmol/L)	10.9 (4.6-64.2)	9.6 (4.0-28.4)	0.11	21.6 (9.9-1727.0)	22.8 (9.1-2887.0)	0.82
PAr	0.28 (0.11-0.62)	0.30 (0.12-0.55)	0.68	0.32 (0.14-0.85)	0.31 (0.17-1.02)	0.86
eGFR (mL/min/1.73m^2)	92.7 (65.7-106.5)	93.3 (70.6-106.3)	0.33	77.2 (47.6-99.8)	76.8 (44.2-106.9)	0.82

eGFR, estimated glomerular filtration rate (<60, moderate to severe renal function loss; 60-89, mild renal function loss; ≥90, normal renal function); PLP, pyridoxal 5'-phosphate; PL, pyridoxal (PL); PA, 4-pyridoxic acid; PAr, PA:(PLP+PL) ratio

[a]2-sided P values were based on t test for normally distributed continuous variables, Mann-Whitney U test for non-normally distributed continuous variables, or chi-square test for categorical variable

Table 9. Spearman correlation coefficients of serum PLP, PL, PA, and PA:(PLP+PL) ratio (PAr) among control subjects of Shanghai Cohort Study (N=258) and the Singapore Chinese Cohort Study (n=104)

	Shanghai cohort			Singapore cohort		
	PL	PA	PAr	PL	PA	PAr
PLP	0.71[b]	0.52[b]	-0.20[a]	0.87[b]	0.66[b]	-0.10
PL		0.51[b]	-0.13[a]		0.77[b]	0.11
PA			0.62[b]			0.58[b]

Abbreviations: PLP, pyridoxal 5'-phosphate; PL, pyridoxal (PL); PA, 4-pyridoxic acid; PAr, PA:(PL+PLP) ratio

[a]$P < 0.05$, [b]$P < 0.0001$

Table 10. Geometric means of serum pyridoxal 5'-phosphate (PLP), pyridoxal (PL), 4-pyridoxic acid (PA), and PA:(PLP+PA) ratio (PAr) in relation to demographic characteristics and lifestyle factors among control subjects, The Shanghai Cohort Study and The Singapore Chinese Health Study

	Shanghai cohort					Singapore cohort				
	N	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr	N	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr
	258	26.4	16.7	11.9	0.26	104	69.2	36.3	39.1	0.32
Smoking status										
Never	113	32.3	18.8	14.4	0.28	63	81.8	45.3	48.0	0.32
Former	16	25.0	18.5	11.6	0.26	23	63.9	36.8	38.4	0.34
Current	129	22.3	14.8	10.0	0.26	18	42.6	16.4	19.6	0.32
P-value		<0.0001	0.02	0.002	0.51		0.005	0.03	0.04	0.90
Among current smokers										
Cigarettes/day										
≤12	58	24.3	16.9	10.8	0.26	8	41.9	12.2	18.2	0.34
13-22	63	21.3	13.3	9.5	0.26	8	42.2	21.8	21.9	0.32
≥23	8	17.8	13.3	9.2	0.30	2	47.3	16.5	17.2	0.26
P-trend		0.09	0.03	0.27	0.36		0.77	0.23	0.84	0.65
Age at blood draw, yr[a]										
45-<55	106	28.5	16.5	10.9	0.24	11	64.8	23.1	22.8	0.26
55-<60	67	27.0	17.2	11.7	0.26	21	63.0	29.1	27.0	0.26
60-<65	85	23.6	16.4	13.3	0.32	22	68.1	32.8	35.8	0.32
≥65	**0**	--	--	--	--	50	73.5	45.9	53.6	0.40
P-trend		0.046	0.97	0.09	<.0001		0.49	0.14	0.03	<0.001
BMI[a,b], kg/m^2										
<18.5	23	23.2	14.8	10.3	0.26	7	49.0	15.2	21.2	0.32
18.5-<23.0	147	25.3	16.5	11.7	0.28	43	69.7	38.9	40.7	0.34
≥23.0†	88	29.3	17.5	12.7	0.26	54	71.9	38.4	41.1	0.32
P-trend		0.053	0.27	0.24	0.84		0.41	0.39	0.48	0.83

Table 10 continued

	Shanghai cohort					Singapore cohort				
	N	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr	N	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr
Level of education[a]										
No formal schooling	13	20.3	12.9	9.4	0.28	21	72.4	44.4	44.8	0.32
Primary school	74	23.8	16.1	11.1	0.28	45	61.2	26.5	30.6	0.32
≥ Secondary	171	28.2	17.3	12.4	0.26	38	78.0	47.1	48.6	0.34
P-trend		0.02	0.13	0.14	0.75		0.57	0.65	0.64	0.61
Alcohol intake, drinks/week[a]										
0	146	25.9	15.7	12.6	0.30	86	70.3	37.4	41.1	0.34
<7	29	28.9	18.5	11.4	0.24	11	62.8	30.2	29.7	0.28
≥7	83	26.5	18.0	10.9	0.24	7	66.0	32.7	33.3	0.32
P-trend		0.77	0.12	0.18	0.001		0.73	0.73	0.56	0.52
Weekly multivitamin use[a]										
No	---	---	---	---	---	93	63.0	30.1	32.3	0.32
Yes	---	---	---	---	---	11	152.2	173.3	196.9	0.52
P-value		---	---	---	---		0.001	<0.001	<0.001	0.005
Diabetes[a]										
No	254	26.2	16.5	11.6	0.26	94	74.5	40.5	41.5	0.32
Yes	4	41.4	28.6	40.4	0.58	10	34.4	12.7	22.6	0.48
P-value		0.16	0.10	0.001	0.003		0.006	0.04	0.23	0.03
eGFR[a,c]										
<60	4	36.1	21.9	21.5	0.36	15	82.7	49.1	90.3	0.46
60-89	102	27.3	17.9	14.2	0.30	60	69.0	39.6	42.7	0.36
≥90	152	25.6	15.8	10.4	0.24	29	63.5	25.8	21.2	0.24
P-trend		0.22	0.03	<0.001	<0.001		0.24	0.03	0.002	<0.001

eGFR, estimated glomerular filtration rate (<60, moderate to severe renal function loss; 60-89, mild renal function loss; ≥90, normal renal function); PLP, pyridoxal 5'-phosphate; PL, pyridoxal (PL); PA, 4-pyridoxic acid; PAr, PA:(PLP+PL) ratio

[a]Geometric means adjusted for smoking

[b]The group with a BMI ≥ 27.5 kg/m^2 was collapsed with the group with a BMI 23-<27.5, because there were only 6 control subjects from the Shanghai cohort and 12 control subjects from the Singapore cohort with a BMI ≥ 27.5 kg/m^2.
[c]Due to the high correlations of PLP and PL with PA, and PA has a high renal clearance. Geometric means of PLP and PL were further adjusted for PA.

Table 11. Associations between serum concentrations of pyridoxal 5'-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid (PA), and PA:(PL+PLP) ratio (PAr) and pancreatic cancer risk in pooled analysis of both cohorts

Biomarkers	Controls	Cases	OR (95%CI)[a]	OR (95%CI)[b]
PLP nmol/L				
<20.0	89	58	1.00	1.00
20.0-29.0	89	42	0.67 (0.4-1.13)	0.68 (0.4-1.15)
29.1-52.4	93	53	0.69 (0.4-1.18)	0.74 (0.42-1.31)
>52.4	91	34	0.41 (0.21-0.78)	0.46 (0.23-0.92)
P-trend			0.01	0.048
PL nmol/L				
<11.8	92	56	1.00	1.00
11.8-16.6	89	58	1.02 (0.63-1.67)	1.18 (0.71-1.97)
16.7-24.0	92	29	0.47 (0.27-0.84)	0.51 (0.28-0.92)
>24.0	89	44	0.74 (0.43-1.27)	0.82 (0.46-1.44)
P-trend			0.06	0.14
PA nmol/L				
<8.8	92	52	1.00	1.00
8.8-13.0	89	56	1.03 (0.63-1.66)	1.19 (0.71-1.97)
13.1-20.4	91	34	0.55 (0.3-0.99)	0.61 (0.33-1.13)
>20.4	90	45	0.69 (0.38-1.25)	0.94 (0.49-1.84)
P-trend			0.09	0.44
PAr				
<0.21	91	48	1.00	1.00
0.21-0.29	90	39	0.79 (0.47-1.35)	0.76 (0.44-1.31)
0.30-0.39	91	53	1.08 (0.67-1.74)	1.22 (0.74-2.04)
>0.39	90	47	0.95 (0.58-1.56)	1.07 (0.63-1.81)
P-trend			0.85	0.48

Abbreviations: PLP, pyridoxal 5'-phosphate; PL, pyridoxal (PL); PA, 4-pyridoxic acid; PAr, PA:(PL+PLP) ratio

[a]Unadjusted odds ratios

[b]Odds ratios were derived from conditional logistic regression models that adjusted for smoking status (never, former, and current smokers), number of alcoholic drinkers per week (continuous), level of education (no formal schooling, primary school, and secondary school or above), history of diabetes (no, yes), and BMI (<18.5, 18.5-<23.0, ≥23.0 kg/m^2). The models including PA, and PAr were further adjusted for estimated glomerular filtration rate.

Table 12. Number of controls and pancreatic cancer cases by serum PLP concentrations in the Shanghai Cohort Study and the Singapore Chinese Cohort Study separately

	Shanghai cohort		Singapore cohort	
	Controls	Cases	Controls	Cases
PLP				
<20.0	84	57	5	1
20.0-29.0	51	26	3	3
29.1-52.4	84	37	23	18
>52.4	39	9	73	36

PLP, pyridoxal 5'-phosphate

Table 13. Associations of cohort-specific quartile concentrations of serum pyridoxal 5'-phosphate (PLP), pyridoxal (PL), 4-pyridoxic acid (PA), and PA:(PL+PLP) ratio (PAr) with pancreatic cancer risk.

	Shanghai cohort					Singapore cohort			
Range	Controls, N=258	Cases, N=129	OR (95%CI)[a]	OR (95%CI)[b]	Range	Controls, N=104	Cases, N=58	OR (95%CI)[a]	OR (95%CI)[b]
PLP									
<18.1	65	47	1.00	1.00	<40.2	26	15	1.00	1.00
18.1-25.7	64	35	0.68 (0.37-1.24)	0.54 (0.29-1.02)	40.2-57.4	26	18	1.17 (0.50-2.77)	1.21 (0.5-2.93)
25.8-35.6	65	23	0.43 (0.22-0.83)	0.39 (0.19-0.80)	57.5-88.2	26	14	0.94 (0.39-2.26)	0.97 (0.38-2.45)
>35.6	64	24	0.45 (0.23-0.87)	0.43 (0.21-0.9)	>88.2	26	11	0.71 (0.25-1.96)	0.75 (0.26-2.15)
P-trend			0.007	0.01				0.51	0.58
PL									
<11.2	65	40	1.00	1.00	<14.3	26	17	1.00	1.00
11.2-15.0	64	37	0.87 (0.47-1.61)	0.93 (0.49-1.78)	14.3-20.0	26	11	0.67 (0.26-1.72)	0.85 (0.30-2.35)
15.1-20.6	65	27	0.62 (0.33-1.18)	0.66 (0.34-1.27)	20.1-41.7	26	17	0.96 (0.41-2.25)	1.20 (0.48-2.99)
>20.6	64	25	0.58 (0.30-1.12)	0.67 (0.33-1.34)	>41.7	26	13	0.76 (0.28-2.03)	0.79 (0.28-2.23)
P-trend			0.06	0.17				0.77	0.93
PA									
<7.6	67	33	1.00	1.00	<15.5	27	18	1.00	1.00
7.6-10.9	62	42	1.36 (0.78-2.38)	1.80 (0.98-3.28)	15.5-21.4	25	9	0.56 (0.22-1.44)	0.64 (0.24-1.69)
11.0-15.5	65	30	0.88 (0.48-1.62)	1.16 (0.59-2.27)	21.5-45.1	26	18	1.01 (0.44-2.29)	1.26 (0.51-3.11)
>15.5	64	24	0.73 (0.38-1.38)	0.98 (0.47-2.01)	>45.1	26	13	0.73 (0.28-1.90)	0.84 (0.3-2.29)
P-trend			0.22	0.82				0.70	0.97
PAr									
<0.21	65	37	1.00	1.00	<0.22	26	12	1.00	1.00
0.21-0.28	64	22	0.56 (0.28-1.1)	0.48 (0.24-0.99)	0.22-0.32	26	21	1.70 (0.7-4.13)	1.81 (0.7-4.67)
0.29-0.36	65	31	0.82 (0.46-1.47)	1.00 (0.53-1.86)	0.33-0.50	26	12	0.95 (0.37-2.45)	1.08 (0.4-2.94)
>0.36	64	39	1.05 (0.6-1.84)	1.32 (0.71-2.44)	>0.50	26	13	0.98 (0.38-2.53)	0.89 (0.32-2.46)
P-trend			0.62	0.20				0.69	0.69

Abbreviations: PLP, pyridoxal 5'-phosphate; PL, pyridoxal (PL); PA, 4-pyridoxic acid; PAr, PA:(PL+PLP) ratio

[a]Odds ratios were derived from conditional logistic regression models that controlled for matching factors including

date of birth (±2 years), date of biospecimen collection (±1 month), and neighborhood of residence at time of enrollment in Shanghai cohort, and age at baseline interview (± 3 years), date of baseline interview (± 2 years), gender, dialect group (Cantonese, Hokkien), and date of biospecimen collection (±6 months) in Singapore cohort.

[b]Odds ratios were derived from conditional logistic regression models that, besides matching factors, included smoking status (never, former, and current smokers), number drinks of alcoholic beverages per week (continuous), level of education (no formal schooling, primary school, and secondary school or above), history of diabetes (no, yes), and BMI (<18.5, 18.5-<23.0, ≥23.0 kg/m^2). The models including PA and PAr were further adjusted for estimated glomerular filtration rate.

Table 14. Epidemiological studies on circulating pyridoxal 5'-phosphate (PLP) and pancreatic cancer risk

Author, year	Country	Age at baseline, sex	No. of cases	Methods of quantifying PLP	Concentration of PLP in controls, nmol/L	OR (95%CI)	Adjustment
Stolzenberg-Solomon, 1999 (111)	Finland	50-69 years, men only	126	Tyrosine decarboxylase apoenzyme method	Median (IQR): 31.9 (22.9-45.9)	T3 vs. T1: 0.48 (0.26–0.88), P for trend = 0.02	Matching factors, serum folate
Schernhammer, 2007 (109)	US	30-84 years, both sexes	208	Vitamin B_6 radioenzymatic assay	Geometric mean (95% CI): 12.7 (11.9-13.6)	Q4 vs. Q1: 0.87 (0.55–1.37), P for trend = 0.37	Matching factors, BMI, physical activity, and diabetes
Chuang, 2011 (110)	Europe	25-70 years, both sexes	463	Mass spectrometry based method	Median (5^{th} – 95^{th} percentile): Men, 35.3 (16.4-98.6) Women, 35.9 (15.6-111.4)	Q5 vs. Q1: 0.7 (0.4-1.1), P for trend not shown	Matching factors, education, smoking, plasma cotinine concentrations, alcohol drinking, BMI, and diabetes

IQR, interquartile range; NCS, nested case-control study; PLP, pyridoxal 5'-phosphate; Q4 vs. Q1: 4^{th} quartile versus 1^{st} quartile; Q5 vs. Q1: 5^{th} quintile versus 1^{st} quintile; T3 vs. T1, 3^{rd} tertile versus 1^{st} tertile.

4.0 SERUM TRYPTOPHAN AND METABOLITES OF THE KYNURENINE PATHWAY AND RISK OF PANCREATIC CANCER IN TWO PROSPECTIVE COHORTS OF ASIAN POPULATIONS

Background: Pyridoxal 5'-phosphate (PLP), the active form of vitamin B_6, may protect against pancreatic cancer development. As functional indicators of PLP, tryptophan and metabolites of the kynurenine (Kyn) pathway may be associated with reduced risk of pancreatic cancer. **Methods:** Two parallel case-control studies including a total of 187 cases and 362 individually matched controls were conducted within the Shanghai Cohort Study and the Singapore Chinese Health Study of 81,501 total participants with over 20 years of follow-up. Using the liquid chromatography-tandem mass spectrometers (LC-MS/MS) method, we quantified tryptophan, six metabolites of the Kyn pathway, and neopterin in serum samples collected from cases prior to cancer diagnosis and matched control subjects. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression models adjusted for covariates. **Results:** Higher serum concentrations of 3'-hydroxyanthranilic acid (HAA) and the ratios of 3'-hydroxyanthranilic acid (HAA):3'-hydroxykynurenine (HK) and HAA:Kyn (markers of the PLP-dependent kynureninase activity) were significantly associated with reduced risk of pancreatic cancer (all *Ps* for trend< 0.05). The ORs (95% CIs) of pancreatic cancer for the highest versus lowest tertile of HAA, the HAA:HK ratio, and the HAA:Kyn ratio were 0.63 (0.39, 1.01), 0.60 (0.37, 0.98), and 0.57 (0.35, 0.92), respectively. Tryptophan, other kynurenines, and neopterin were not associated with risk of pancreatic cancer. **Conclusion:** Our findings for inverse associations with HAA and the ratios of HAA:HK and HAA:Kyn suggest

that sufficient intracellular levels of PLP for the use in the Kyn pathway may protect against the development of pancreatic cancer.

4.1 INTRODUCTION

Pancreatic cancer is among the deadliest malignancies in the world. In the US, the 5-year survival rate of pancreatic cancer was 7.7% during the year 2006-2012, ranking the lowest among all common cancers (220). Few prevention strategies are available for pancreatic cancer, given that only 34-39% of pancreatic cancer can be attributed to the two established modifiable risk factors, smoking and obesity(221, 222). Studies are needed to identify novel risk or protective factors for pancreatic cancer in order to develop tools to monitor high-risk populations and intervention trials to reduce risk of pancreatic cancer.

Pyridoxal 5'-phosphate (PLP), the active form of vitamin B_6, plays a role in multiple mechanisms that may modulate carcinogenesis, including DNA methylation and synthesis, antioxidant defense system, and inflammation (152). Using two parallel Asian nested case-control studies, we recently found higher concentrations of pyridoxal 5'-phosphate (PLP), the active form of vitamin B_6, in the serum were associated with reduced risk of pancreatic cancer. Compared with PLP <20 nmol/L, the odds ratio (OR) and 95% confidence interval (CI) for > 52.4 nmol/L was 0.46 (0.23, 0.92) (P trend=0.048). However, low concentration of PLP in the circulation may not indicate low availability of PLP within cells but instead may indicate an increase in cellular PLP uptake (223). Therefore, the role of intracellular PLP deficiency in the development of pancreatic cancer remains unclear.

Indoleamine 2,3-dioxygenase (IDO) catalyzes breakdown of tryptophan to kynurenine (Kyn), the initial step of the Kyn pathway of the tryptophan metabolism (224) (Figure 3). Kyn can be further metabolized to kynurenic acid (KA) by kynurenine amino transferase (KAT). Alternatively, Kyn can be converted to 3-hydroxykynurenine (HK), and HK can be further converted to xanthurenic acid (XA) by KAT or 3-hydroxyanthranilic acid (HAA) by KYNU. KYNU also catalyzes the direct conversion of Kyn to HAA, with the production of anthranilic acid (AA) as an intermediate (224). Given that PLP is the coenzyme for KAT and KYNU, biomarkers of the Kyn pathway and their ratios have been used as indicators of intracellular functional status of PLP (123).

Interferon-γ (IFN-γ) can upregulate the Kyn pathway via activation of indoleamine 2,3-dioxygenase (IDO). In addition, IFN-γ stimulates the synthesis of neopterin by activated macrophages (225). Kyn:tryptophan ratio (KTR) and neopterin, as biomarkers of IFN-γ induced immune activation (225), were associated with increased risk of lung and colorectal cancers (138, 226). However, the associations of KTR and neopterin with risk of pancreatic cancer have not been evaluated in epidemiological studies. A number of kynurenines, such as Kyn, 3'-hydroxykynurenine (HK), and 3'-hydroxyanthranilic acid (HAA) have immunoregulatory properties (227) and can interact with aryl hydrocarbon receptor (AHR) (228, 229). AHR is implicated in pancreatic carcinogenesis (132). The associations between metabolites of the Kyn pathway and risk of pancreatic cancer are unknown.

The purpose of the current study was to evaluate whether tryptophan and metabolites of the Kyn pathway, as functional indicators of PLP and immunoregulatory compounds, are associated with risk of developing pancreatic cancer in two case-control studies nested within two prospective cohorts of Asian populations.

4.2 METHODS

4.2.1 Study subjects

The Shanghai Cohort Study consisted of 18,244 Chinese men aged 45 to 64 years at enrollment (1986-1989) (204). At the time of recruitment, trained interviewers conducted face-to-face interview at participants' home and asked for information on demographics, height, body weight, use of tobacco and alcohol, and medical history. Each participant donated a non-fasting blood sample and a spot urine sample immediately following the interview. All collected biospecimens were kept on ice (at around 4 °C) before they were processed and aliquots of serum and urine specimens have been stored at -80 °C until laboratory analysis.

The Singapore Chinese Health Study consisted of 63,257 Chinese men and women aged 45-74 years at enrollment (1993-1998) (159). At recruitment, participants were interviewed face-to-face in their home by a trained interviewer using a structured questionnaire which asked for information on demographics, height, weight, use of tobacco and alcohol, physical activity, medical history, usual dietary intake, dietary supplemental use, and family history of cancer. Non-fasting blood samples and spot urine samples were collected from a 3% random sample of cohort members between 1994 and 1999, and extended to all surviving cohort members between 2000 and 2005. By April 2005, blood and/or urine specimens were collected from 32,543 participants, representing a consent rate of 60%. Serum and urine specimens were kept in insulated boxes with ice (4 °C) until processing, and stored at -80 °C. For Singapore subjects, blood sample was collected on average 6.5 (range, 1.2-11.0) years after the baseline interview. Follow-up I interview (N=52,322) was administered during 1999-2003 and the consent rate reached over 90% among surviving cohort members.

Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the Shanghai Cancer Institute, the National University of Singapore, and the University of Pittsburgh.

4.2.2 Case ascertainment and control selection

Incident pancreatic cancer cases and deaths from the Shanghai cohort were identified through annual in-person interviews of all surviving cohort participants and routine review of reports from the population-based Shanghai Cancer Registry. As of the most recent follow-up in 2015, 3.7% of original cohort participants were lost to the follow-up interview and 3.3% declined the continued follow-up interview. The diagnosis of all incident cancer cases was confirmed via review of medical records. As of December 31st 2009, the cut-off date for the present study, 129 incident cases of pancreatic cancer [International Classification of Disease (ICD)-9 code, 157] were identified among participants of the Shanghai cohort.

The incident cancer cases and deaths among cohort members of the Singapore cohort were identified through routine record linkage with databases of the Singapore National Birth and Death Registry and National Cancer Registry (173). In the Singapore cohort, less than 1% of original cohort members were lost to follow-up due to their migration out of Singapore. As of December 31st, 2013, 58 incident pancreatic cancer cases (ICD-Oncology code, C25) were identified among participants of the Singapore cohort who had available serum samples.

For each case, two control subjects were randomly selected among all eligible participants who were free of cancer at the time of cancer diagnosis of the index case within the same cohort. To be consistent with the matching criteria used in previous nested case-control

studies in the Shanghai cohort, controls were matched to the index case on date of birth (±2 years), date of biospecimen collection (±1 month), and neighborhood of residence at time of enrollment (207). In the Singapore cohort, cases and controls were matched on age at baseline interview (± 3 years), date of baseline interview (± 2 years), gender, dialect group (Cantonese, Hokkien), and date of biospecimen collection (±6 months).

4.2.3 Assessment of serum biomarkers

For each subject, 60 μL serum was pulled from the biorepository. Serum tryptophan, Kyn, KA, XA, AA, HK, HAA, neopterin, and creatinine were measured by liquid chromatography-tandem mass spectrometers (LC-MS/MS) using the methods described previously (208). Serum PLP was measured in a previous study. All biochemical analyses were performed at Bevital A/S (www.bevital.no) at Bergen, Norway. Serum specimens of cases and their matched controls were processed, aliquoted, shipped in insulated boxes with dry ice, and assayed together in the same batch. Laboratory technicians were blinded to case-control status of the test samples. For quality control purposes, 14 duplicatedsamples (2% of testing samples) from a pooled serum sample collected from ineligible study subjects were included in 7 batches (2 duplicated samples per batch). The within-batch coefficients of variation (CVs) for the biomarkers ranged from 0.90% for tryptophan to 5.53% for XA, and the between-batch CVs ranged from 1.12% for tryptophan to 14.70% for XA (**Table 15**).

4.2.4 Statistical analysis

The ratios of KA:Kyn and XA:HK were calculated as indicators of the activity of the PLP-dependent enzyme KAT. Likewise, HAA:HK and HAA:Kyn were calculated as indicators of the PLP-dependent enzyme KYNU. All four ratios of kynurenines indicate the functional status of PLP. KTR and neopterin were used as biomarkers of IFN-γ-induced immune activation.

We logarithmically transformed raw values of all biomarkers and their ratios to normalize their skewed distributions towards high values. Coefficient of partial determination (partial R^2 of Spearman correlation coefficient) of PLP with tryptophan and kynurenines were calculated to indicate the variability of these biomarkers that can be explained by PLP while controlling for cohort and gender. The associations of PLP with tryptophan and kynurenines were evaluated using Analysis of Covariance (ANCOVA) after adjustment with cohort and gender.

Study subjects were divided into tertiles based on the distribution of individual biomarkers among controls pooled from two cohorts. Odds ratios (ORs) and their 95% confidence intervals (CIs) of pancreatic cancer for tertiles of the biomarkers were calculated using conditional logistic regression (209). Ordinal values (e.g., 1, 2, and 3) for each of the biomarkers were used for testing linear trend in the biomarker-pancreatic cancer risk association models. Stratified analyses by cohort were performed using tertile variables based on control subjects of two cohorts pooled. Heterogeneity in the biomarker-pancreatic cancer risk association between two cohorts was assessed by calculating a p-value for comparing the odds ratios of pancreatic cancer for tertile variables of biomarkers (as ordinal values) between two cohorts (211).

The multivariable conditional logistic regression models included following reported risk factors for pancreatic cancer as potential confounders: body mass index (BMI) (<18.5, 18.5-<23, ≥23 kg/m^2), level of education (no formal schooling, primary school, secondary school and above), smoking status (never smokers, former smokers, current smokers), alcohol consumption (number of drinks per week), history of physician-diagnosed diabetes (no, yes), estimated glomerular filtration rate (eGFR) (212), and cohort study (Shanghai, Singapore).

In Shanghai cohort, all the covariate variables were based on baseline interview. In the Singapore cohort, the information on smoking and diabetes were derived mainly from follow-up I interview (98%) supplemented by baseline interview (2%), since the status of smoking and diabetes may change over time. A validation study of the incident diabetes cases in the Singapore cohort observed that 99% of individuals who reported a history of diabetes were considered valid cases (205). Another study analyzed percentage of hemoglobin A1c (HbA1c) (glycated hemoglobin) in blood samples among individuals who reported being free of diabetes at baseline and follow-up interview and found 94.4% of those individuals were below the HbA1c threshold for diabetes (206). Other demographic and lifestyle factors used were derived from the baseline interview only.

To study whether the associations of kynurenines with risk of pancreatic cancer were independent from PLP, multivariable logistic regression models were further adjusted for PLP (<20, 20-29.0, 29.1-52.4, >52.4 nmol/L). In addition, we examined potential joint effects between PLP (<20, ≥ 20 nmol/L) and kynurenines on risk of pancreatic cancer. PLP <20 nmol/L (the cutpoint for vitamin B_6 deficiency (210)) and the lowest tertiles of each biomarker were chosen as the joint reference category. To assess if the association between a biomarker and risk of pancreatic cancer was modified by PLP, p value for interaction was calculated. To reduce the

potential effect of disease progression on the concentrations of tryptophan and kynurenines, we repeated the analysis after excluding cases diagnosed within 2 years after blood draw and their matched controls.

Statistical analyses were carried out using SAS software version 9.3 (SAS Institute, Cary, NC). All *P* values reported are two-sided, and those that were <0.05 were considered to be statistically significant.

4.3 RESULTS

The mean age at blood draw for study participants of the Shanghai and Singapore cohorts was 56.4 and 63.4 years, respectively. The average (range) time between blood draw and cancer diagnosis was 12.5 years (3 months to 23.2 years) for cases of the Shanghai cohort, and 6.8 (5 months to 13.0 years) for cases of the Singapore cohort.

Detailed baseline characteristics of pancreatic cancer cases and matched controls in the Shanghai and Singapore cohorts were presented previously. Briefly, subjects who developed pancreatic cancer were more likely to smoke cigarettes at baseline in the Shanghai cohort, whereas the distribution of smoking status was similar among cases and control subjects in the Singapore cohort. The distributions of BMI, level of education, alcohol intake, and history of diabetes were comparable between cases and control subjects in both cohorts. Pancreatic cancer cases had lower concentrations of HAA, and the ratios of HAA:HK and HAA:Kyn compared with control subjects (**Table 16**). Geometric means of serum tryptophan and kynurenines among cases and control subjects by cohort can be found in table 17 (**Table 17**).

There was a trend of geometric means of tryptophan, HAA, and ratios of XA:HK, HAA:HK, and HAA:Kyn by increasing concentration of PLP (**Table 18**). The HAA:HK ratio had the strongest correlation with concentration of PLP. PLP accounted for 11% of variability of the HAA:HK ratio (R^2=0.138), followed by the XA:HK ratio (R^2=0.108), HAA and the HAA:Kyn ratio (R^2=0.061).

HAA and the ratios of HAA:HK and HAA:Kyn were inversely associated with risk of pancreatic cancer (all P values for trend ≤0.04) (**Table 19**). Compared with the lowest tertile, the second and third tertiles of HAA and the ratios of HAA:HK and HAA:Kyn were associated with a 40% to 50% reduced risk of pancreatic cancer. In addition, the second tertile of the XA:HK ratio was associated with a statistically significant 47% reduced risk of pancreatic cancer, but the association attenuated and lost statistical significance with the third versus first tertile (P for trend>0.05). In contrast to most kynurenines, the second tertile of AA was associated with a statistically significant 2-fold increase in risk of pancreatic cancer, though the linear trend was not significant. Tryptophan, Kyn, KA, HK, XA, and the ratio of KA: Kyn were not associated with risk of pancreatic cancer. The associations of tryptophan and kynurenines with risk of pancreatic cancer were similar between the Shanghai cohort and the Singapore cohort (**Table 21 and 22**).

After further adjustment for PLP, the inverse associations of HAA and the HAA:HK ratio with risk of pancreatic cancer were attenuated and lost statistical significance (data not shown), whereas the inverse association of the HAA:Kyn ratio remained statistically significant. The ORs (95%CIs) for the second and third versus first tertiles of HAA:Kyn were 0.59 (0.37-0.93) and 0.59 (0.37-0.96) (P for trend=0.02).

The inverse associations of Kyn, HK, HAA, and the ratios of KA:Kyn, HAA:HK, and HAA:Kyn with risk of pancreatic cancer were more apparent among PLP deficient individuals (<20 nmol/L), compared with those whose PLP were 20 nmol/L or higher (**Table 23**). Among PLP deficient individuals, the highest tertiles of biomarker levels were associated with a 70% to 80% reduced risk of pancreatic cancer compared with the lowest tertile. PLP modified the associations of HK and HAA:Kyn ratio with risk of pancreatic cancer (P for interaction <0.05). There was no statistically significant association of KA, AA, XA, and XA:HK with risk of pancreatic cancer in the joint analyses with PLP. No association was observed between biomarkers of IFN-γ-induced immune activation, KTR and neopterin, and risk of pancreatic cancer (**Table 24**).

After excluding cases diagnosed within two years of serum collection and their matched control subjects, the inverse associations of HAA, and ratios of HAA:HK and HAA:Kyn with risk of pancreatic cancer were strengthened. Compared with the lowest tertiles, OR (95%CI) for highest tertiles of HAA, , and ratios of HAA:HK and HAA:Kyn were 0.55 (0.33-0.90) (P for trend=0.01), 0.55 (0.33-0.92) (P for trend=0.02), and 0.52 (0.31-0.86) (P for trend=0.006), respectively.

4.4 DISCUSSION

We report statistically significant inverse associations between the ratios of HAA:HK and HAA:Kyn, indicators of functional status of PLP, and risk of pancreatic cancer after adjustment with BMI, smoking, alcohol intake, and history of diabetes in a study of 187 pancreatic cancer cases and 362 matched control subjects from two prospective cohorts of Asian populations. This

finding supported our previous observation that higher concentrations of serum PLP were associated with reduced risk of pancreatic cancer. In addition, we made a novel observation that HAA, an anti-inflammatory compound, in serum samples collected an average of 12.5 (Shanghai cohort) and 6.8 (Singapore cohort) years before cancer diagnosis was inversely associated with reduced risk of pancreatic cancer. The highest tertiles of the ratios of HAA:HK and HAA:Kyn and HAA were associated with about 40% reduced risk of pancreatic cancer, compared with the lowest tertiles. The inverse associations of HAA, and the ratio of HAA:HK and HAA:Kyn were strengthened after excluding cases diagnosed within the first two years of serum collection and their matched controls, indicating the associations were not due to impact of underlying disease on the kynurenine metabolism.

Our study was the first to evaluate the associations between metabolites of the Kyn pathway and their ratios and risk of pancreatic cancer. Our study was also the first to evaluate the associations between ratios of metabolites of the Kyn metabolite, as functional surrogate of vitamin B_6 status, and risk of any cancer. PLP plays an important role in the Kyn pathway as the coenzyme for KYNU and KAT. In a human feeding trial, dietary vitamin B_6 restriction resulted in reduction of KA and elevation of HK in the circulation (120). In the current study, we found that higher ratios of HAA:HK and HAA:Kyn, indicators of KYNU activity, were associated with reduced risk of pancreatic cancer, whereas the ratios of KA:Kyn and XA:HK, indicators of KAT activity, were not associated with risk of pancreatic cancer. The discrepancy between the indicators of KYNU and KAT activity may be explained by the fact that KYNU is more susceptible to PLP deficiency than KAT (230). Therefore, the ratios of HAA:HK and HAA:Kyn may be better markers of functional status of PLP than the ratios of KA:Kyn and XA:HK. The current findings of inverse associations of the ratios of HAA:HK and HAA:Kyn with risk of

pancreatic cancer supported our previous observation on circulating PLP. Interestingly, among PLP deficient individuals, the subgroup with higher ratios of HAA:HK and HAA:Kyn were at reduced risk of developing pancreatic cancer compared to those with lower ratios of HAA:HK and HAA:Kyn. In other words, our findings suggest that individuals who had low levels of circulating PLP but high levels of intracellular functional PLP had lower risk of developing pancreatic cancer. Therefore, intracellular functional status of PLP, indicated by the ratios of HAA:HK and HAA:Kyn, could be more relevant in pancreatic cancer development than circulating PLP alone. Future studies on PLP and disease risk should consider including biomarkers of functional status of PLP, such as the ratios of HAA:HK and HAA:Kyn to better assess pancreatic cancer risk.

We report that HAA, a metabolite of kynurenine, was inversely associated with risk of pancreatic cancer. The inverse association between HAA and risk of pancreatic cancer is biological plausible. HAA has strong anti-inflammatory properties demonstrated by its ability to inhibit the production of pro-inflammatory IL-17 (231). IL-17 was involved in the initiation and progression of preinvasive pancreatic neoplasia in genetically engineered mice, (232). Therefore, higher levels of HAA may protect against the development of pancreatic cancer through inhibiting the production of IL-17. In addition, HAA is a precursor of an endogenous ligand to the aryl hydrocarbon receptor (AHR) (229) that when activated resulted in cell cycle arrest and growth inhibition of pancreatic cancer cell lines through induction of the cyclin-dependent kinase inhibitor p21(132).

IFN-γ activates the breakdown of tryptophan to Kyn catalyzed by IDO (224). In addition, IFN-γ stimulates the synthesis of neopterin, a metabolites of of guanosine triphosphate (GTP), by activated monocyte-derived macrophages and dendritic cells (225). In a case-control study

nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) of 893 lung cancer cases, higher KTR was associated with increased risk of lung cancer (226). The OR and 95%CI comparing the fifth versus the first quintile was 1.36 (0.96-1.92) (P for trend = 0.009). Another prospective cohort analysis of 6594 Norweigian men and women (971 cancer cases) found positive associations of KTR and neopterin with overall cancer risk (138). The hazard ratios (HRs) and 95%CIs associated with per SD increment were 1.07 (1.01-1.14) for KTR, and 1.09 (1.03-1.16) for neopterin. In analysis on major cancer types (e.g., colorectal cancer, breast cancer, prostate cancer, and lung cancer), KTR and neopterin were only associated with risk of colorectal cancer (N=175), suggesting the associations of KTR and neopterin with cancer risk may be cancer-specific. Our study is the first to evaluate KTR and neopterin, as biomarkers of IFN-γ-induced immune activation, in relation to risk of pancreatic cancer, and we found no association. More studies are needed to elucidate the associations of KTR and neopterin with risk of pancreatic cancer.

To the best of our knowledge, this is the first epidemiological study that evaluated tryptophan and metabolites of the Kyn pathway and risk of pancreatic cancer. The strengths of the current study include prospective design, a long follow-up, and a comprehensive measurement of tryptophan and kynurenines using an accurate and reliable mass-spectrometry-based assay. In addition, a previous study found age and renal function were important determinants of biomarkers of the Kyn metabolic pathway (233). The matched case-control design (e.g., matched on age and sex) of the present study and adjustment for BMI, smoking, history of diabetes, and renal function in the statistical analysis minimized potential confounding effects of these factors on the associations of tryptophan and kynurenines with risk of pancreatic

cancer. Our study has a modest sample size. Larger studies are needed to validate our results on tryptophan and the kynurenines in other populations.

In conclusion, the present study reported inverse associations between higher ratios of HAA:HK and HAA:Kyn, as indicators of functional status of PLP, and risk of pancreatic cancer. These associations were more apparent among PLP-deficient individuals. This finding supported our previous observation of an inverse association between circulating PLP and risk of pancreatic cancer, and further indicates intracellular functional status of PLP may be more relevant in evaluating pancreatic cancer risk compared with circulating PLP levels alone. In addition, higher concentrations of HAA, an anti-inflammatory metabolite from the Kyn pathway, was associated with reduced risk of pancreatic cancer. This finding sheds light on the potential etiological role of HAA in pancreatic cancer development. Experimental studies in animal models are needed to investigate the direct effect of HAA and other Kyn metabolites on pancreatic cancer progression. HAA and the ratios of HAA:HK and HAA:Kyn have the potential to be developed into biomarkers to assess pancreatic cancer risk in the general population.

4.5 TABLES

Table 15. Within-batch and between-batch coefficients of variations (CV) of tryptophan and metabolites of the kynurenine pathway, among controls of Shanghai and Singapore cohorts pooled (N=362)

	Within-batch CV, %	Between-batch CV, %
Tryptophan, μmol/L	0.9	1.1
Kynurenine, nmol/L	1.4	4.4
AA, nmol/L	5.5	6.4
KA, nmol/L	5.0	5.9
HK, nmol/L	4.2	3.8
XA, nmol/L	5.5	14.7
HAA, nmol/L	4.7	10.2
Neopterin	5.4	7.3

[a]Abbreviations: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid

Table 16. Geometric means (95%CI) [a] of biomarkers of tryptophan and the kynurenine pathway among cases and controls in the Shanghai and Singapore cohorts

	Controls, N=362	Cases, N=187	P
Tryptophan, µmol/L	71.2 (69.3-73.0)	70.6 (68.4-72.8)	0.62
Kynurenine, nmol/L	1540 (1480-1580)	1500 (1460-1560)	0.41
AA, nmol/L	19.5 (18.1-21.0)	20.0 (18.3-21.9)	0.57
KA, nmol/L	53.6 (50.4-56.9)	51.7 (48.1-55.7)	0.36
HK, nmol/L	44.3 (42.1-46.6)	43.5 (40.9-46.2)	0.54
XA, nmol/L	16.0 (15.0-17.1)	15.3 (14.1-16.6)	0.26
HAA, nmol/L	37.3 (35.1-40.0)	34.8 (32.4-37.5)	0.07
Product substrate ratios of B_6 enzymes			
KAT			
KA:Kyn [b]	3.50 (3.32-3.68)	3.42 (3.22-3.64)	0.54
XA:HK	0.36 (0.34-0.38)	0.35 (0.33-0.38)	0.55
KYNU			
AA:Kyn [b]	1.28 (1.20-1.38)	1.32 (1.22-1.44)	0.45
HAA:HK	0.84 (0.80-0.89)	0.80 (0.75-0.86)	0.12
HAA:Kyn [b]	2.44 (2.31-2.58)	2.30 (2.15-2.46)	0.08
IFN-γ-induced inflammatory biomarkers			
KTR [b]	2.26 (2.14-2.40)	2.38 (2.22-2.56)	0.26
Neopterin	25.4 (23.5-27.5)	27.2 (24. 6-30.2)	0.30

Acronyms: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KAT: Kynurenine aminotransferase; KTR, ratio of kynurenine to tryptophan; KYNU: Kynureninase; XA, xanthurenic acid;

[a] Adjusted for gender and cohort

[b] The numbers should be multiplied by 10^{-2}

Table 17. Baseline demographic characteristics, lifestyle factors, and biomarkers of tryptophan and the kynurenine pathway among pancreatic cancer cases and control subjects, The Shanghai Cohort Study and The Singapore Chinese Health Study [a]

	Shanghai cohort			Singapore cohort		
	Controls	Cases	P [b]	Controls	Cases	P [b]
N	258	129		104	58	
Age at interview, mean (SD), years	56.4 (5.5)	56.5 (5.5)	0.74	57.1 (7.2)	57.9 (7.5)	0.51
Age at blood draw, mean (SD), years	56.4 (5.5)	56.5 (5.5)	0.74	64.0 (7.1)	64.9 (7.6)	0.47
BMI, mean (SD), kg/m^2	21.9 (2.8)	22.5 (3.0)	0.08	23.1 (3.2)	23.2 (3.6)	0.79
Female (%)	0	0		39.4	39.7	0.98
Education level (%)			0.36			0.42
No formal schooling	5.0	2.3		20.2	12.1	
Primary school	28.7	26.4		43.3	48.3	
Secondary school or above	66.3	71.3		36.5	39.7	
Smoking status (%)			0.003			0.87
Never	43.8	27.1		60.6	58.6	
Former	6.2	4.7		22.1	20.7	
Current	50.0	68.2		17.3	20.7	
Alcohol intake, drinks/week (%)			0.74			0.61
0	56.6	54.3		82.7	87.9	
<7	11.2	14.0		10.6	8.6	
≥7	32.2	31.8		6.7	3.5	
Diabetes (%)			0.52			0.88
No	98.5	99.2		90.4	89.7	
Yes	1.55	0.78		9.6	10.3	

Table 17 continued

	Shanghai cohort			Singapore cohort		
	Controls	Cases	P [b]	Controls	Cases	P [b]
Weekly multivitamin use (%)			---			0.69
No	---	---		89.4	91.4	
Yes	---	---		10.6	8.62	
Serum biomarker concentrations (geometric means, 95%CI)						
PLP, nmol/L	26.4 (24.4-28.7)	23.3 (20.8-26.2)	0.08	70.9 (60.39-83.3)	61. 8 (49.9-766)	0.31
Tryptophan, μmol/L	76.4 (74.8-78.0)	76.5 (74.3-78.8)	0.95	69.9 (67.4-72.6)	67.9 (64.7-71.4)	0.36
Kynurenine, nmol/L	1560 (1540-1600)	1540 (1500-1600)	0.54	1600 (1520-1660)	1560 (1480-1660)	0.58
AA, nmol/L	19.7 (18.5-21.1)	20.8 (18.9-22.9)	0.37	22.4 (20.6-24.3)	21.6 (19.3-24.2)	0.63
KA, nmol/L	52.9 (50.3-55.6)	51.1 (47.6-54.8)	0.43	56.8 (52.3-61.7)	55.0 (49.2-61.4)	0.64
HK, nmol/L	45.5 (43.5-47.6)	45.5 (42.7-48.5)	0.998	45.9 (43.4-48.5)	43.2 (40.1-46.5)	0.19
XA, nmol/L	17.2 (16.3-18.3)	17.0 (15.6-18.4)	0.76	16.6 (15.4-17.9)	14.8 (13.3-16.4)	0.08
HAA, nmol/L	35.8 (33.9-37.9)	33.8 (31.3-36.6)	0.24	41.9 (39.4-44.5)	38.1 (35.2-41.4)	0.07
Product substrate ratios of B_6 enzymes						
KAT						
KA:Kyn [c]	3.38 (3.24-3.52)	3.30 (3.10-3.52)	0.55	3.6 (3.3-3.8)	3.5 (3.2-3.9)	0.83
XA:HK	0.38 (0.36-0.40)	0.38 (0.35-0.40)	0.90	0.36 (0.34-0.39)	0.34 (0.31-0.37)	0.31
KYNU						
AA:Kyn [c]	1.26 (1.18-1.34)	1.34 (1.22-1.46)	0.34	1.40 (1.30-1.52)	1.38 (1.26-1.54)	0.82
HAA:HK	0.79 (0.75-0.83)	0.74 (0.69-0.80)	0.15	0.91 (0.86-0.97)	0.88 (0.81-0.96)	0.55
HAA:Kyn [c]	2.30 (2.18-2.42)	2.18 (2.02-2.34)	0.22	2.64 (2.48-2.78)	2.44 (2.26-2.64)	0.12
IFN-γ-induced inflammatory biomarkers						
KTR [c]	2.06 (2.00-2.10)	2.02 (1.96-2.10)	0.52	2.28 (2.16-2.40)	2.30 (2.16-2.46)	0.83
Neopterin	14.4 (13.8-14.9)	13.5 (12.8-14.2)	0.06	24.9 (23.4-26. 4)	26.1 (24.0-28.3)	0.36
eGFR (mL/min/1.73m^2)	88.8 (87.3-90.4)	90.8 (88.7-93.1)	0.14	76.5 (73.2-80.0)	77.4 (72.9-82.1)	0.76

[a] Abbreviations: AA, anthranilic acid; eGFR, estimated glomerular filtration rate (<60, moderate to severe renal function loss; 60-89, mild renal function loss; ≥90, normal renal function); HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic

acid; KAT: Kynurenine aminotransferase; Kyn, kynurenine; KYNU: Kynureninase; PLP, pyridoxal 5'-phosphate; XA, xanthurenic acid

[b] 2-sided P values were based on t-test for continuous variables or chi-square test for categorical variable

[c] The numbers should be multiplied by 10^{-2}

Table 18. Geometric means [a] of biomarkers of tryptophan and the kynurenine pathway by PLP in quartile in controls of Shanghai and Singapore cohorts pooled (N=362) [b]

	PLP, nmol/L					
	<20	20-<29.0	29.0-<52.4	≥52.4	P for trend	R^2 with PLP [c]
N	90	90	92	90		
Tryptophan, µmol/L	64.7	70.1	71.2	73.0	<0.001	0.052
Kynurenine, nmol/L	1520	1560	1500	1540	0.98	<0.01
AA, nmol/L	18.8	17.1	19.2	21.3	0.13	0.010
KA, nmol/L	52.2	53.3	51.1	60.5	0.11	0.021
HK, nmol/L	50.4	46.7	44.9	41.4	<0.001	0.016
XA, nmol/L	15.1	15.9	16.5	17.1	0.08	0.026
HAA, nmol/L	33.4	35.4	37.3	40.7	0.004	0.061
Product substrate ratios of B_6 enzymes						
KAT						
KA:Kyn [d]	3.42	3.44	3.40	3.92	0.06	0.025
XA:HK	0.30	0.34	0.37	0.41	<0.001	0.108
KYNU						
AA:Kyn [d]	1.24	1.10	1.28	1.38	0.10	0.010
HAA:HK	0.68	0.76	0.82	0.98	<0.001	0.138
HAA:Kyn [d]	2.20	2.29	2.48	2.65	0.003	0.061

[a] Geometric means adjusted for gender and cohort

[b] Abbreviations: AA, anthranilic acid; eGFR, estimated glomerular filtration rate (<60, moderate to severe renal function loss; 60-89, mild renal function loss; ≥90, normal renal function); HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KAT: Kynurenine aminotransferase; Kyn, kynurenine; KYNU: Kynureninase; PLP, pyridoxal 5'-phosphate; XA, xanthurenic acid

[c] Partial R^2 between tryptophan metabolites and their ratios and PLP (continuous) adjusted for cohort and gender

[d] The numbers should be multiplied by 10^{-2}

Table 19. Associations between biomarkers of tryptophan and the kynurenine pathway and pancreatic cancer risk, Shanghai and Singapore cohorts pooled [a]

Biomarkers	T1		T2		T3		P for trend
	Co/Ca	R [b] (ref)	Co/Ca	OR (95%CI) [b]	Co/Ca	OR (95%CI) [b]	
Tryptophan	120/66	1.00	123/63	0.90 (0.57-1.40)	119/58	0.88 (0.55-1.40)	0.58
Kynurenine	120/76	1.00	123/56	0.69 (0.44-1.09)	119/55	0.70 (0.42-1.16)	0.14
AA	117/47	1.00	120/77	2.02 (1.22-3.34)	116/59	1.51 (0.87-2.64)	0.17
KA	120/69	1.00	123/64	0.99 (0.62-1.57)	119/54	0.82 (0.51-1.32)	0.42
HK	119/68	1.00	118/62	0.92 (0.58-1.44)	116/53	0.69 (0.42-1.12)	0.14
XA	120/73	1.00	124/58	0.81 (0.53-1.25)	118/56	0.82 (0.52-1.29)	0.37
HAA	117/79	1.00	120/52	0.61 (0.38-0.97)	116/52	0.63 (0.39-1.01)	0.04
Product substrate ratios of B_6 enzymes							
KAT							
KA:Kyn	120/74	1.00	123/57	0.71 (0.45-1.11)	119/56	0.78 (0.49-1.25)	0.27
XA:HK	117/64	1.00	120/67	0.53 (0.32-0.88)	116/52	0.85 (0.53-1.36)	0.53
KYNU							
AA:Kyn	117/56	1.00	120/62	1.17 (0.72-1.88)	116/65	1.27 (0.76-2.12)	0.36
HAA:HK	117/77	1.00	120/58	0.64 (0.40-1.02)	116/48	0.60 (0.37-0.98)	0.04
HAA:Kyn	117/83	1.00	120/50	0.59 (0.37-0.93)	116/50	0.57 (0.35-0.92)	0.01

[a] Abbreviations: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KAT: Kynurenine aminotransferase; Kyn, kynurenine; KYNU: Kynureninase; XA, xanthurenic acid

[b] Adjusted for education (no schooling, primary school, secondary school and higher), BMI (<18.5, 18.5-<23.0, ≥23.0), smoking status (never, former, current), alcohol drinking (No. alcoholic drinks per week), diabetes, eGFR (continuous), and cohort

Table 20. Range of tryptophan and metabolites of the kynurenine pathway, among controls of Shanghai and Singapore cohorts pooled (N=362)

	Range		
	T1	T2	T3
Tryptophan, μmol/L	<69.7	69.7-80.1	>80.1
Kynurenine, nmol/L	<1453	1453-1718	>1718
AA, nmol/L	<16.4	16.4-23.0	>23.0
KA, nmol/L	<46.4	46.4-63.5	>63.5
HK, nmol/L	<39.5	39.5-52.6	>52.6
XA, nmol/L	<14.7	14.7-21.3	>21.3
HAA, nmol/L	<33.2	33.2-45.0	>45.0
Product substrate ratios of B_6 enzymes			
KAT			
KA:Kyn	<2.97	2.97-3.99	>3.99
XA:HK	<0.33	0.33-0.45	>0.45
KYNU			
AA:Kyn	<1.07	1.07-1.43	>1.43
HAA:HK	<0.74	0.74-0.99	>0.99
HAA:Kyn	<2.14	2.14-2.86	>2.86

[a] Abbreviations: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KAT: Kynurenine aminotransferase; Kyn, kynurenine; KYNU: Kynureninase; XA, xanthurenic acid

Table 21. Associations between biomarkers of tryptophan and the kynurenine pathway and pancreatic cancer risk, in Shanghai cohort [a]

Biomarkers	T1		T2		T3		P trend
	Co/Ca	OR (ref) [b]	Co/Ca	OR (95%CI) [a]	Co/Ca	OR (95%CI) [a]	
Tryptophan	66/40	1.00	94/42	0.69 (0.39-1.19)	98/47	0.72 (0.41-1.25)	0.25
Kynurenine	87/55	1.00	92/42	0.75 (0.44-1.29)	79/32	0.64 (0.35-1.18)	0.14
AA	97/39	1.00	80/49	2.23 (1.22-4.06)	72/37	1.62 (0.85-3.09)	0.14
KA	89/50	1.00	92/42	0.93 (0.54-1.63)	77/37	0.90 (0.51-1.58)	0.71
HK	88/46	1.00	80/42	1.04 (0.60-1.80)	81/37	0.76 (0.43-1.34)	0.34
XA	83/44	1.00	88/41	1.02 (0.59-1.76)	87/44	1.01 (0.59-1.73)	0.98
HAA	95/57	1.00	81/33	0.65 (0.37-1.13)	73/35	0.78 (0.45-1.36)	0.31
Product substrate ratios of B_6 enzymes							
KAT							
KA:Kyn	94/53	1.00	74/35	0.87 (0.50-1.52)	90/41	0.79 (0.46-1.38)	0.41
XA:HK	78/50	1.00	84/28	0.50 (0.27-0.94)	87/47	0.87 (0.50-1.50)	0.69
KYNU							
AA:Kyn	98/45	1.00	81/37	1.14 (0.65-2.02)	70/43	1.56 (0.85-2.85)	0.16
HAA:HK	95/61	1.00	80/35	0.64 (0.37-1.10)	74/29	0.62 (0.35-1.09)	0.08
HAA:Kyn	95/63	1.00	75/30	0.64 (0.38-1.10)	79/32	0.56 (0.32-0.97)	0.03

[a].Abbreviations: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KAT: Kynurenine aminotransferase; Kyn, kynurenine; KYNU: Kynureninase; XA, xanthurenic acid

[b] Adjusted for education (no schooling, primary school, secondary school and higher), BMI (<18.5, 18.5-<23.0, ≥23.0 kg/m^2), smoking status (never, former, current), alcohol drinking (No. alcoholic drinks per week), diabetes, and eGFR (continuous)

Table 22. Associations between biomarkers of tryptophan and the kynurenine pathway and pancreatic cancer risk, in Singapore cohort [a]

Biomarkers	T1		T2		T3		P trend	P [b]
	Co/Ca	OR [c] (ref)	Co/Ca	OR (95%CI) [c]	Co/Ca	OR (95%CI) [c]		
Tryptophan	54/26	1.00	29/21	1.55 (0.69-3.44)	21/11	1.18 (0.45-3.09)	0.60	0.29
Kynurenine	33/21	1.00	31/14	0.62 (0.26-1.48)	40/23	0.89 (0.32-2.47)	0.80	0.60
AA	20/8	1.00	40/28	1.77 (0.63-4.98)	44/22	1.19 (0.37-3.85)	0.93	0.52
KA	31/19	1.00	31/22	1.08 (0.45-2.61)	42/17	0.59 (0.23-1.54)	0.29	0.47
HK	31/22	1.00	38/20	0.69 (0.30-1.57)	35/16	0.49 (0.18-1.33)	0.15	0.44
XA	37/29	1.00	36/17	0.55 (0.25-1.21)	31/12	0.40 (0.14-1.11)	0.053	0.09
HAA	22/22	1.00	39/19	0.43 (0.18-1.04)	43/17	0.30 (0.11-0.81)	0.02	0.11
Product substrate ratios of B_6 enzymes								
KAT								
KA:Kyn	26/21	1.00	49/22	0.53 (0.24-1.19)	29/15	0.65 (0.25-1.70)	0.34	0.67
XA:HK	39/27	1.00	36/16	0.64 (0.27-1.49)	29/15	0.79 (0.31-2.03)	0.60	0.81
KYNU								
AA:Kyn	19/11	1.00	39/25	0.96 (0.37-2.53)	46/22	0.72 (0.25-2.04)	0.44	0.16
HAA:HK	22/16	1.00	40/23	0.64 (0.24-1.70)	42/19	0.52 (0.18-1.50)	0.24	0.85
HAA:Kyn	22/20	1.00	45/20	0.45 (0.18-1.12)	37/18	0.49 (0.18-1.29)	0.13	0.81

[a] Abbreviations: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KAT: Kynurenine aminotransferase; Kyn, kynurenine; KYNU: Kynureninase; XA, xanthurenic acid

[b] P value for heterogeneity comparing Shanghai cohort with Singapore cohort

[c] Adjusted for education (no schooling, primary school, secondary school and higher), BMI (<18.5, 18.5-<23.0, ≥23.0 kg/m^2), smoking status (never, former, current), alcohol drinking (No. alcoholic drinks per week), diabetes, and eGFR (continuous)

Table 23. Joint analysis of biomarkers of tryptophan and the kynurenine pathway with PLP on pancreatic cancer risk, Shanghai and Singapore cohorts pooled [a]

	PLP < 20 nmol/L		PLP ≥ 20 nmol/L		P for interaction
	Co/Ca	OR(95%CI) [b]	Co/Ca	OR(95%CI) [b]	
Kynurenine					0.06
T1	26/25	1.00 (Ref)	94/51	0.46 (0.22-0.97)	
T2	34/22	0.61 (0.26-1.45)	89/34	0.33 (0.15-0.71)	
T3	29/11	0.31 (0.11-0.83)	90/44	0.43 (0.20-0.96)	
HK					0.02
T1	24/22	1.00 (Ref)	95/46	0.42 (0.20-0.88)	
T2	21/18	0.84 (0.33-2.11)	97/44	0.40 (0.18-0.86)	
T3	38/15	0.26 (0.10-0.67)	78/38	0.41 (0.18-0.90)	
HAA					0.07
T1	39/33	1.00(ref)	78/46	0.52 (0.26-1.03)	
T2	27/15	0.51 (0.22-1.21)	93/37	0.34 (0.16-0.71)	
T3	17/7	0.27 (0.09-0.81)	99/45	0.42 (0.21-0.85)	
KA:Kyn					0.10
T1	33/26	1.00(ref)	87/48	0.53 (0.27-1.04)	
T2	27/21	0.74 (0.34-1.62)	96/36	0.35 (0.17-0.73)	
T3	29/11	0.36 (0.14-0.94)	90/45	0.54 (0.26-1.09)	
HAA:HK					0.23
T1	37/34	1.00(ref)	80/49	0.46 (0.23-0.93)	
T2	27/16	0.50 (0.22-1.16)	93/34	0.30 (0.14-0.63)	
T3	19/5	0.17 (0.05-0.53)	97/45	0.37 (0.18-0.77)	
HAA:Kyn					0.01
T1	37/34	1.00(ref)	80/49	0.54 (0.27-1.06)	
T2	27/16	0.61 (0.28-1.36)	93/34	0.32 (0.16-0.64)	
T3	19/5	0.24 (0.08-0.73)	94/45	0.40 (0.20-0.78)	

[a] Abbreviations: AA, anthranilic acid; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KAT: Kynurenine aminotransferase; Kyn, kynurenine; KYNU: Kynureninase; XA, xanthurenic acid

[b] Adjusted for education (no schooling, primary school, secondary school and higher), BMI (<18.5, 18.5-<23.0, ≥23.0), smoking status (never, former, current), alcohol drinking (No. alcoholic drinks per week), diabetes, and eGFR (continuous)

Table 24. Associations between IFN-γ-induced inflammatory biomarkers and pancreatic cancer risk, Shanghai and Singapore cohorts pooled [a]

Biomarkers	T1		T2		T3		P trend
	Co/Ca	OR [b] (ref)	Co/Ca	OR (95%CI) [b]	Co/Ca	OR (95%CI) [b]	
KTR	120/65	1.00	123/67	0.97 (0.6-1.57)	119/55	0.88 (0.52-1.49)	0.64
Neopterin	117/69	1.00	121/49	0.70 (0.41-1.18)	115/65	0.91 (0.48-1.72)	0.69

[a] Abbreviations: KTR, kynurenine:tryptophan ratio

[b] Adjusted for education (no schooling, primary school, secondary school and higher), BMI (<18.5, 18.5-<23.0, ≥23.0 kg/m^2), smoking status (never, former, current), alcohol drinking (No. alcoholic drinks per week), diabetes, eGFR (continuous), and cohort

Table 25. Associations between IFN-γ-induced inflammatory biomarkers and pancreatic cancer risk, in Shanghai cohort [a]

Biomarkers	T1		T2		T3		P trend
	Co/Ca	OR [b] (ref)	Co/Ca	OR (95%CI) [b]	Co/Ca	OR (95%CI) [b]	
KTR	35/21	1.00	35/17	0.82 (0.36-1.88)	34/20	1.05 (0.42-2.65)	0.97
Neopterin[c]	35/19	1.00	35/16	0.85 (0.36-1.99)	34/23	1.46 (0.60-3.54)	0.41

[a] Abbreviations: KTR, kynurenine:tryptophan ratio

[b] Adjusted for education (no schooling, primary school, secondary school and higher), BMI (<18.5, 18.5-<23.0, ≥23.0 kg/m^2), smoking status (never, former, current), alcohol drinking (No. alcoholic drinks per week), diabetes, eGFR (continuous)

[c] Tertiles were created based on distribution of neopterin among control subjects from the Shanghai cohort alone, since the distribution of neopterin in the Shanghai cohort and the Singapore cohort were very different.

Table 26. Associations between IFN-γ-induced inflammatory biomarkers and pancreatic cancer risk, in Singapore cohort [a]

Biomarkers	T1		T2		T3		P trend
	Co/Ca	OR [b] (ref)	Co/Ca	OR (95%CI) [b]	Co/Ca	OR (95%CI) [b]	
KTR	86/49	1.00	87/41	0.87 (0.48-1.56)	85/39	0.91 (0.49-1.69)	0.78
Neopterin[c]	83/57	1.00	85/34	0.61 (0.34-1.12)	81/34	0.67 (0.36-1.24)	0.21

[a] Abbreviations: KTR, kynurenine:tryptophan ratio

[b] Adjusted for education (no schooling, primary school, secondary school and higher), BMI (<18.5, 18.5-<23.0, ≥23.0 kg/m^2), smoking status (never, former, current), alcohol drinking (No. alcoholic drinks per week), diabetes, eGFR (continuous)

[c] Tertiles were created based on distribution of neopterin among control subjects from the Singapore cohort alone, since the distribution of neopterin in the Shanghai cohort and the Singapore cohort were very different.

5.0 GENERAL DISCUSSION

5.1 SUMMARY OF FINDINGS

The overall aim of this dissertation was to evaluate dietary one-carbon metabolism-related nutrients (e.g., betaine, choline, folate, methionine, and vitamins B_2, B_6, and B_{12}), serum B_6 vitamers (e.g. PLP, PL, and PA) and metabolites in the PLP-dependent kynurenine pathway in relation to risk of pancreatic cancer.

One-carbon metabolism plays an important role in DNA synthesis and methylation. Vitamin B_6 and folate are important enzymatic cofactors. Choline, betaine, and methionine are major sources of methyl groups (234). Betaine, acquired from diet or produced via choline oxidation pathway, is a substrate of the regeneration of methionine from homocysteine (Figure 1). Methionine is a precursor of S-adenosylmethionine (SAM), an important methyl donor in DNA methylation. A majority of epidemiologic studies that evaluated one-carbon metabolism-related compounds in relation to pancreatic cancer risk have focused on folate. Several pooled analyses of cohort studies in US and Europe reported a null association between dietary or circulating folate and pancreatic cancer risk (65, 109, 110). Few studies investigated the potential role of one-carbon metabolism-related compounds other than folate in the risk of pancreatic cancer. Dietary methionine was associated with lower risk of pancreatic cancer in a Swedish study (102). There have been no previous epidemiologic studies of choline and betaine on

pancreatic cancer risk. Due to the complexity in one-carbon metabolism, in the first prospective cohort study, I comprehensively evaluated dietary intake of seven one-carbon metabolism-related compounds in relation to pancreatic cancer risk. A barrier to studies on dietary choline and betaine and pancreatic cancer risk was the absence of data on content of choline and betaine in food. Food content information of choline and betaine has recently become available and were added into the Singapore Food Composition Database (167-172). The principal findings of the first study were statistically significant inverse associations between dietary vitamin B_6 and choline and pancreatic cancer risk (published in (235)). These novel results suggest a role of one-carbon metabolism in pancreatic carcinogenesis.

The finding on dietary vitamin B_6 was further supported by follow-up biomarker studies. Pyridoxal-5'-phosphate (PLP) is the active form of vitamin B_6 and serves as an enzymatic cofactor in the synthesis of nucleic acids, amino acids (i.e., tryptophan), and cellular antioxidants (107, 236). Pyridoxal (PL, the form taken up by tissues) and 4-pyridoxic acid (PA, a major catabolite of PLP) are other major forms of B_6 vitamers in blood. In addition, the ratios of metabolites of the Kyn pathway can be served as markers of intracellular functional status of PLP (123). Except for plasma PLP, no epidemiological study investigated the association between B_6 vitamers and functional measures of PLP (ratios of metabolites of the Kyn pathway) and pancreatic cancer risk. A barrier to those studies has been lack of reliable and accurate biochemical assays for those compounds. Using a mass-spectrometry based method with high-throughput capability, we have quantified a panel of B_6 vitamers and metabolites of the Kyn pathway. This comprehensive approach will significantly advance our understanding of the relationships of vitamin B_6 and kynurenines with risk of pancreatic cancer, individually and in combination (237). In two case-control studies nested within two prospective cohorts of Asian

populations, PLP greater than 52.4 nmol/L was associated with 54% reduced risk of pancreatic cancer compared with PLP deficient individuals; the second and third tertiles of HAA:HK ratio and HAA:Kyn ratio, as biomarkers of intracellular functional status of PLP, were associated with about 40% reduced risk of pancreatic cancer compared with the lowest tertiles.

5.2 PUBLIC HEALTH SIGNIFICANCE

In the U.S., pancreatic cancer is the fourth leading cause of cancer-related death in both men and women(238). The five-year survival rate of pancreatic cancer patients after cancer diagnosis is less than 8%, due in part to the lack of effective treatments at the late stage of diagnosis; ~80% of cancers have spread to regional lymph nodes or distant metastasis (26). As a highly lethal disease, little is known about the risk factors for pancreatic cancer. Cigarette smoking and obesity are the only established modifiable risk factors for pancreatic cancer (146, 147). Findings of the study on dietary intake and serum biomarkers of diet-related factors will contribute to the understanding of pancreatic cancer etiology. The identified risk biomarkers (e.g. PLP, HAA, and ratios of HAA:HK and HAA:Kyn) can be applied for risk assessment and early detection of pancreatic cancer. Therefore, findings of the study will provide desirable information to enrich individuals with risk characteristics who would be beneficial from screening and early detection of pancreatic cancer.

Eventually, findings of the studies (e.g., dietary intake of vitamin B_6 and choline, PLP, and ratios of kynurenines) can be used to develop a risk assessment model to identify individuals at high risk of developing pancreatic cancer. In addition, clinical trials can be designed to evaluate the chemopreventive effect of dietary supplementation with vitamin B_6, choline and/or

other one-carbon metabolism-related compounds, and kynurenines such as HAA on the changes of molecular and cellular marker of pancreatic pathogenesis, and eventually for primary prevention of pancreatic cancer in high risk population (31).

In addition, the study provided evidence for a protective role of vitamin B_6 against pancreatic cancer development. In Shanghai and Singapore cohorts, the population attributable risk (PAR) of low PLP status (by cohort-specific quartiles), smoking status, and BMI was calculated using a multivariate approach:

$$PAR = 1 - \sum_i \frac{pei}{RRi}$$

Where i indexes the mutually exclusive strata formed by a specific potential risk factor, pei is the proportion of cases in each stratum, and RRi is the relative risk associated with each stratum (239). For example, in Shanghai cohort, the PAR for low PLP calculated as $1 - \frac{\frac{number\ of\ cases\ in\ the\ 1st\ quartile}{odds\ ratio\ associated\ with\ the\ 1st\ quartile} + \ldots + \frac{number\ of\ cases\ in\ the\ 4th\ quartile}{odds\ ratio\ associated\ with\ the\ 4th\ quartile}}{total\ number\ of\ cases}$, that is $1 - (\frac{47}{2.272} + \frac{35}{1.274} + \frac{23}{0.873} + \frac{24}{1})/129$. In Shanghai cohort, the PAR for low PLP status, smoking status, and BMI was 23.6%, 46.3%, and 16.0%, respectively. In Singapore cohort, the corresponding figures were 18.9%, 13.6%, and 14.5%. In addition to pancreatic cancer, vitamin B_6 was reported to be associated with reduced risk of lung and colorectal cancers (240, 241). Vitamin B_6 deficiency is also associated with other health issues such as neurological disorders and skin changes. Fortification of B vitamins into grain products such as flour and cereals has been implemented in the US since 1941 (242). Given that Asian populations may have a relatively high percentage of vitamin B_6 deficient individuals, food fortification of vitamin B_6 can be considered as a public health approach to reduce deficiency and improve health at the population level. Selection of formulated foods can be based on major staples consumed by Asian populations such as rice

instead of cereals, given that Asian populations have different dietary patterns from US and European populations.

According to a pancreatic cancer progression model based on genetic evolution of pancreatic cancer, pancreatic cancer takes on average 20 years to develop from the first mutation until patient death (243). Given that most cases live less than a year after diagnosis (median survival <6 months) and it takes on average 2.7 years from the metastasis to cancer death, most cases are diagnosed after the tumor gains metastatic ability. Due to the deep abdomen location of pancreas and limitation of current diagnostic methods, primary and secondary preventive measures that can prevent early-stage tumors from progressing into more advanced malignancies can be valuable methods to reduce pancreatic cancer mortality. Serum sample were collected on average 12.5 years prior to diagnosis in the Shanghai cohort and 6.8 years prior to diagnosis in the Singapore cohort. Based on the model, samples in Shanghai cases could be collected during the precursor stages when the lesions had not gained invasive ability and samples in Singapore cases could be collected during the stages when the lesions had not gain metastatic ability. Therefore, the findings of present studies on compounds such as PLP and HAA are relevant to early-stage development of pancreatic cancer. If the potential protective effects of PLP and HAA are confirmed in mechanistic studies, intervention trials could be designed to develop chemopreventive agents against pancreatic cancer.

5.3 STRENGTHS AND LIMITATIONS

The strengths of the proposed study include novel hypothesis, prospective design, a comprehensive dietary assessment of one-carbon metabolism-related nutrients, and a high resolution and accurate mass-spectrometry-based approach to measure biomarkers of B_6 vitamers and metabolites of the Kyn pathway.

Due to the prospective nature of the study, the first prospective cohort study was able to eliminate the opportunity of differential recall bias. The study populations in the two prospective cohort studies, Shanghai Cohort Study and Singapore Chinese Health Study, have distinct dietary habits and environmental exposures from each other and from Western populations. Despite numerous epidemiological studies that have investigated diet- or biomarker-pancreatic cancer risk associations in Western populations, data from Asian populations are sparse. The comparison of exposure-disease associations from two cohorts takes advantage of differences in the distribution of the exposure variable across two cohorts. Given the similar incidence rates of pancreatic cancer across Singapore, Shanghai, and the U.S. populations (**Table 1**), the study population used for the proposed study is appropriate and the findings may be relevant to the U.S. population. The proposed study will likely contribute to the literature in novel risk or preventive factors for pancreatic cancer in populations with different levels of dietary or *in vivo* exposures. Given pancreatic cancer has an extremely high mortality and few known risk factors, study findings from the proposed study could have important public health implications.

The proposed study may also be challenged by several issues. First, some risk factors for pancreatic cancer such as smoking and obesity may impact the fluxes of pathways in one-carbon metabolism and the Kyn pathway, and thus confound the relationship between concentrations of

biomarkers in those metabolic pathways and pancreatic cancer risk. To overcome this issue, smoking status and obesity were controlled in the statistical analysis. Furthermore, stratified analysis were performed by smoking status and BMI levels to eliminate or minimize their confounding effect. Second, B_6 vitamers were measured at the systemic level using serum specimens and might not reflect the changes of biomarkers in the local environment in the pancreas. However, we overcame this issue by measuring metabolites of the Kyn pathway and evaluated ratios of kynurenines, as surrogates of intracellular functional status of PLP, in relation to risk of pancreatic cancer in the third study. In the second and third studies, though lab measurement errors are possible sources of misclassification, we have a number of procedures in place to limit the opportunity for systematic bias. For example, case-control sets will be measured together in lab batches, and the lab personnel will be blinded as to the case-control status of the specimens. We included 2% repeated samples to evaluate the within- and between-batch variation in biomarker measurements.

In the third study, we did not observe an association of neopterin and KTR with pancreatic cancer risk. In addition to cytokine-induced IDO, TDO is constitutively expressed in liver and can also catalyze the production of kynurenine from tryptophan. Therefore, KTR is a less specific marker for cellular immunity activation compared to neopterin. It was reported that neopterin is sensitive to sunlight and the level of neopterin may decrease over time(244). However, the effect of decay in neopterin will likely to impact the specimens from cases and controls in the same way, and therefore may bias the results towards null results.

5.4 FUTURE DIRECTION

Since PLP and biomarkers of intracellular functional activity of PLP were found to be inversely associated with risk of pancreatic cancer, I plan to conduct animal experiments, in collaboration with laboratory scientists, to confirm chemopreventive effect of vitamin B_6 on the inhibition of pancreatic carcinogenesis. Such data are crucial to confirm the biological mechanism of action and further development of vitamin B_6 as chemopreventive agents against pancreatic cancer in humans.

Furthermore, higher choline intake was associated with reduced risk of pancreatic cancer. I plan to study biomarkers of choline and other methyl donors such as folate, methionine and betaine in relation to pancreatic cancer. Levels of one-carbon metabolism-related compounds are inter-dependent. For example, choline and folate can complement each other in supplying C1 units in regeneration of methionine (**Figure 1**). Varied levels of dietary folate, and vitamins B_6 and/or B_{12} altered the requirements of choline in human and rats (245, 246). In contrast to most previous studies that only evaluated the associations between single one-carbon metabolism-related compounds and cancer risk, I plan to use a pathway analysis approach to evaluate the aggregate effect of one-carbon metabolism-related compounds on pancreatic cancer risk.

In addition to the role in DNA synthesis and methylation, one-carbon metabolism branches out with amino acid metabolism (i.e., serine and glycine) (247), choline metabolism (248) and transsulfuration pathway (production of glutathione) (249) that are related to detoxification of carcinogens and the inhibition of carcinogenesis. PLP, plays a central role in enzyme activities in multiple pathways of one-carbon metabolism and amino acid metabolism (e.g., tryptophan). A pathway analysis approach can integrate biological pathways of one-carbon metabolism and metabolism of related amino acids (e.g., serine, glycine, tryptophan, etc.).

Pathway analysis will help pinpoint the most important biological pathway driving the observed associations between serum one-carbon metabolism-related compounds and pancreatic cancer risk.

BIBLIOGRAPHY

1. Ferlay J SI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. http://globocan.iarc.fr/ ed. Lyon, France: International Agency for Research on Cancer; 2013.

2. Wahi MM, Shah N, Schrock CE, Rosemurgy AS, 2nd, Goldin SB. Reproductive factors and risk of pancreatic cancer in women: a review of the literature. Ann Epidemiol. 2009;19:103-11.

3. Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. Nat Rev Gastroenterol Hepatol. 2009;6:699-708.

4. International Agency for Research on Cancer. IARC Monographs : The known causes of human cancer by organ site. 2014; Available from: http://monographs.iarc.fr/

5. Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, et al. Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. JAMA. 2014;311:183-92.

6. Weiderpass E, Partanen T, Kaaks R, Vainio H, Porta M, Kauppinen T, et al. Occurrence, trends and environment etiology of pancreatic cancer. Scand J Work Environ Health. 1998;24:165-74.

7. World Cancer Research Funk / American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Pancreatic Cancer2012.

8. American Cancer Society. Cancer Facts & Figures 2016. Atlanta: American Cancer Society; 2016.

9. American Cancer Society. Cancer Facts & Figures 2014. Atlanta: American Cancer Society; 2014.

10. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective Washington, DC: American Institute of Cancer Research; 2007.

11. Surveillance, Epidemiology, and End Results (SEER) Program (http://www.seer.cancer.gov/) SEER*Stat Database: Incidence - SEER 9 Regs Research Data, Nov 2013 Sub (1973-2011). National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch; April 2013, based on the November 2013 submission.

12. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 2014;74:2913-21.

13. DevCan: Probability of Developing or Dying of Cancer Software, Version 6.6.1. . Statistical Research and Applications Branch, National Cancer Institute; 2012.

14. Wang L, Yang GH, Lu XH, Huang ZJ, Li H. Pancreatic cancer mortality in China (1991-2000). World J Gastroenterol. 2003;9:1819-23.

15. Shen J, Goyal A, Sperling L. The emerging epidemic of obesity, diabetes, and the metabolic syndrome in china. Cardiol Res Pract. 2012;2012:178675.

16. Ansary-Moghaddam A, Huxley R, Barzi F, Lawes C, Ohkubo T, Fang X, et al. The effect of modifiable risk factors on pancreatic cancer mortality in populations of the Asia-Pacific region. Cancer Epidemiol Biomarkers Prev. 2006;15:2435-40.

17. Pourhoseingholi MA, Vahedi M, Baghestani AR. Burden of gastrointestinal cancer in Asia; an overview. Gastroenterol Hepatol Bed Bench. 2015;8:19-27.

18. International Agency for Research on Cancer. World Cancer Report 2008. Lyon, France: IARC; 2008.

19. Hariharan D, Saied A, Kocher HM. Analysis of mortality rates for pancreatic cancer across the world. HPB (Oxford). 2008;10:58-62.

20. Khan SA, Taylor-Robinson SD, Toledano MB, Beck A, Elliott P, Thomas HC. Changing international trends in mortality rates for liver, biliary and pancreatic tumours. J Hepatol. 2002;37:806-13.

21. Tominaga S, Kuroishi T. Epidemiology of pancreatic cancer. Semin Surg Oncol. 1998;15:3-7.

22. Kato I, Kuroishi T, Tominaga S. Descriptive epidemiology of subsites of cancers of the liver, biliary tract and pancreas in Japan. Jpn J Clin Oncol. 1990;20:232-7.

23. Zhang J, Dhakal I, Yan H, Phillips M, Kesteloot H, Registries SC. Trends in pancreatic cancer incidence in nine SEER Cancer Registries, 1973-2002. Ann Oncol. 2007;18:1268-79.

24. Lopez AD CN, Piha T A descriptive model of the cigarette epidemic in developed countries. Tobacco Control. 1994;3:242-7.

25. Shafey O DS, Guindon GE (eds),. Tobacco control country profiles 2003, second edition. Atlanta, GA: American Cancer Society; 2003.

26. Gong Z, Holly EA, Bracci PM. Survival in population-based pancreatic cancer patients: San Francisco Bay area, 1995-1999. Am J Epidemiol. 2011;174:1373-81.

27. Sener SF, Fremgen A, Menck HR, Winchester DP. Pancreatic cancer: a report of treatment and survival trends for 100,313 patients diagnosed from 1985-1995, using the National Cancer Database. J Am Coll Surg. 1999;189:1-7.

28. Dawood S, Broglio K, Gonzalez-Angulo AM, Buzdar AU, Hortobagyi GN, Giordano SH. Trends in survival over the past two decades among white and black patients with newly diagnosed stage IV breast cancer. J Clin Oncol. 2008;26:4891-8.

29. Howlader N NA, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z,Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds),. SEER Cancer Statistics Review, 1975-2011, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2011/, based on November 2013 SEER data submission, posted to the SEER web site, April 2014.

30. Sun H, Ma H, Hong G, Sun H, Wang J. Survival improvement in patients with pancreatic cancer by decade: a period analysis of the SEER database, 1981-2010. Sci Rep. 2014;4:6747.

31. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet. 2004;363:1049-57.

32. Longnecker DS, Karagas MR, Tosteson TD, Mott LA. Racial differences in pancreatic cancer: comparison of survival and histologic types of pancreatic carcinoma in Asians, blacks, and whites in the United States. Pancreas. 2000;21:338-43.

33. Lynch SM, Vrieling A, Lubin JH, Kraft P, Mendelsohn JB, Hartge P, et al. Cigarette smoking and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. Am J Epidemiol. 2009;170:403-13.

34. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best Pract Res Clin Gastroenterol. 2006;20:197-209.

35. Lowenfels AB, Maisonneuve P. Epidemiology and prevention of pancreatic cancer. Jpn J Clin Oncol. 2004;34:238-44.

36. Malfertheiner P, Schutte K. Smoking--a trigger for chronic inflammation and cancer development in the pancreas. Am J Gastroenterol. 2006;101:160-2.

37. Wittel UA, Pandey KK, Andrianifahanana M, Johansson SL, Cullen DM, Akhter MP, et al. Chronic pancreatic inflammation induced by environmental tobacco smoke inhalation in rats. Am J Gastroenterol. 2006;101:148-59.

38. U.S. Department of Health and Human Services. The Health Consequences of Smoking—50 Years of Progress. A Report of the Surgeon General. . Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014.

39. Arslan AA, Helzlsouer KJ, Kooperberg C, Shu XO, Steplowski E, Bueno-de-Mesquita HB, et al. Anthropometric measures, body mass index, and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). Arch Intern Med. 2010;170:791-802.

40. Genkinger JM, Spiegelman D, Anderson KE, Bernstein L, van den Brandt PA, Calle EE, et al. A pooled analysis of 14 cohort studies of anthropometric factors and pancreatic cancer risk. Int J Cancer. 2011;129:1708-17.

41. Jiao L, Berrington de Gonzalez A, Hartge P, Pfeiffer RM, Park Y, Freedman DM, et al. Body mass index, effect modifiers, and risk of pancreatic cancer: a pooled study of seven prospective cohorts. Cancer Causes Control. 2010;21:1305-14.

42. Berrington de Gonzalez A, Sweetland S, Spencer E. A meta-analysis of obesity and the risk of pancreatic cancer. Br J Cancer. 2003;89:519-23.

43. Larsson SC, Orsini N, Wolk A. Body mass index and pancreatic cancer risk: A meta-analysis of prospective studies. Int J Cancer. 2007;120:1993-8.

44. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008;371:569-78.

45. Roberts DL, Dive C, Renehan AG. Biological mechanisms linking obesity and cancer risk: new perspectives. Annu Rev Med. 2010;61:301-16.

46. Bracci PM. Obesity and pancreatic cancer: overview of epidemiologic evidence and biologic mechanisms. Mol Carcinog. 2012;51:53-63.

47. Raimondi S, Lowenfels AB, Morselli-Labate AM, Maisonneuve P, Pezzilli R. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. Best Pract Res Clin Gastroenterol. 2010;24:349-58.

48. Habbe N, Langer P, Sina-Frey M, Bartsch DK. Familial pancreatic cancer syndromes. Endocrinol Metab Clin North Am. 2006;35:417-30, xi.

49. Couch FJ, Johnson MR, Rabe KG, Brune K, de Andrade M, Goggins M, et al. The prevalence of BRCA2 mutations in familial pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2007;16:342-6.

50. Chang MC, Wong JM, Chang YT. Screening and early detection of pancreatic cancer in high risk population. World J Gastroenterol. 2014;20:2358-64.

51. Breast Cancer Linkage C. Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst. 1999;91:1310-6.

52. Carriere C, Young AL, Gunn JR, Longnecker DS, Korc M. Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. Biochem Biophys Res Commun. 2009;382:561-5.

53. Carriere C, Young AL, Gunn JR, Longnecker DS, Korc M. Acute pancreatitis accelerates initiation and progression to pancreatic cancer in mice expressing oncogenic Kras in the nestin cell lineage. PLoS One. 2011;6:e27725.

54. Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, et al. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell. 2007;11:291-302.

55. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144:1252-61.

56. Hassan MM, Bondy ML, Wolff RA, Abbruzzese JL, Vauthey JN, Pisters PW, et al. Risk factors for pancreatic cancer: case-control study. Am J Gastroenterol. 2007;102:2696-707.

57. Lowenfels AB, Maisonneuve P, Whitcomb DC, Lerch MM, DiMagno EP. Cigarette smoking as a risk factor for pancreatic cancer in patients with hereditary pancreatitis. JAMA. 2001;286:169-70.

58. Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. N Engl J Med. 1993;328:1433-7.

59. Pedrazzoli S, Pasquali C, Guzzinati S, Berselli M, Sperti C. Survival rates and cause of death in 174 patients with chronic pancreatitis. J Gastrointest Surg. 2008;12:1930-7.

60. Malka D, Hammel P, Maire F, Rufat P, Madeira I, Pessione F, et al. Risk of pancreatic adenocarcinoma in chronic pancreatitis. Gut. 2002;51:849-52.

61. Karlson BM, Ekbom A, Josefsson S, McLaughlin JK, Fraumeni JF, Jr., Nyren O. The risk of pancreatic cancer following pancreatitis: an association due to confounding? Gastroenterology. 1997;113:587-92.

62. Rocca G, Gaia E, Iuliano R, Caselle MT, Rocca N, Calcamuggi G, et al. Increased incidence of cancer in chronic pancreatitis. J Clin Gastroenterol. 1987;9:175-9.

63. Bracci PM, Wang F, Hassan MM, Gupta S, Li D, Holly EA. Pancreatitis and pancreatic cancer in two large pooled case-control studies. Cancer Causes Control. 2009;20:1723-31.

64. Goldacre MJ, Wotton CJ, Yeates D, Seagroatt V, Collier J. Liver cirrhosis, other liver diseases, pancreatitis and subsequent cancer: record linkage study. Eur J Gastroenterol Hepatol. 2008;20:384-92.

65. Bao Y, Michaud DS, Spiegelman D, Albanes D, Anderson KE, Bernstein L, et al. Folate intake and risk of pancreatic cancer: pooled analysis of prospective cohort studies. J Natl Cancer Inst. 2011;103:1840-50.

66. Bulathsinghala P, Syrigos KN, Saif MW. Role of vitamin d in the prevention of pancreatic cancer. J Nutr Metab. 2010;2010:721365.

67. Skinner HG, Michaud DS, Giovannucci E, Willett WC, Colditz GA, Fuchs CS. Vitamin D intake and the risk for pancreatic cancer in two cohort studies. Cancer Epidemiol Biomarkers Prev. 2006;15:1688-95.

68. Genkinger JM, Wang M, Li R, Albanes D, Anderson KE, Bernstein L, et al. Dairy products and pancreatic cancer risk: a pooled analysis of 14 cohort studies. Ann Oncol. 2014;25:1106-15.

69. Zhang J, Dhakal IB, Gross MD, Lang NP, Kadlubar FF, Harnack LJ, et al. Physical activity, diet, and pancreatic cancer: a population-based, case-control study in Minnesota. Nutr Cancer. 2009;61:457-65.

70. Anderson KE, Kadlubar FF, Kulldorff M, Harnack L, Gross M, Lang NP, et al. Dietary intake of heterocyclic amines and benzo(a)pyrene: associations with pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2005;14:2261-5.

71. Li D, Day RS, Bondy ML, Sinha R, Nguyen NT, Evans DB, et al. Dietary mutagen exposure and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev. 2007;16:655-61.

72. Stolzenberg-Solomon RZ, Cross AJ, Silverman DT, Schairer C, Thompson FE, Kipnis V, et al. Meat and meat-mutagen intake and pancreatic cancer risk in the NIH-AARP cohort. Cancer Epidemiol Biomarkers Prev. 2007;16:2664-75.

73. Zhu J, Rashid A, Cleary K, Abbruzzese JL, Friess H, Takahashi S, et al. Detection of 2-amino-1-methyl-6-phenylimidazo [4,5-b]-pyridine (PhIP)-DNA adducts in human pancreatic tissues. Biomarkers. 2006;11:319-28.

74. IARC. International Agency for Research on Cancer handbooks of cancer prevention. Fruit and vegetables. Lyon (France): IARC Press. 2003.

75. Koushik A, Spiegelman D, Albanes D, Anderson KE, Bernstein L, van den Brandt PA, et al. Intake of fruits and vegetables and risk of pancreatic cancer in a pooled analysis of 14 cohort studies. Am J Epidemiol. 2012;176:373-86.

76. Vrieling A, Verhage BA, van Duijnhoven FJ, Jenab M, Overvad K, Tjonneland A, et al. Fruit and vegetable consumption and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2009;124:1926-34.

77. Potter JD. Pancreas cancer--we know about smoking, but do we know anything else? Am J Epidemiol. 2002;155:793-5; discussion 6-7.

78. Lyon JL, Slattery ML, Mahoney AW, Robison LM. Dietary intake as a risk factor for cancer of the exocrine pancreas. Cancer Epidemiol Biomarkers Prev. 1993;2:513-8.

79. Zhou BF, Stamler J, Dennis B, Moag-Stahlberg A, Okuda N, Robertson C, et al. Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom, and United States in the late 1990s: the INTERMAP study. J Hum Hypertens. 2003;17:623-30.

80. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willett WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. Am J Clin Nutr. 2000;72:912-21.

81. Key TJ, Schatzkin A, Willett WC, Allen NE, Spencer EA, Travis RC. Diet, nutrition and the prevention of cancer. Public Health Nutr. 2004;7:187-200.

82. Genkinger JM, Spiegelman D, Anderson KE, Bergkvist L, Bernstein L, van den Brandt PA, et al. Alcohol intake and pancreatic cancer risk: a pooled analysis of fourteen cohort studies. Cancer Epidemiol Biomarkers Prev. 2009;18:765-76.

83. Michaud DS, Vrieling A, Jiao L, Mendelsohn JB, Steplowski E, Lynch SM, et al. Alcohol intake and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium (PanScan). Cancer Causes Control. 2010;21:1213-25.

84. Gapstur SM, Jacobs EJ, Deka A, McCullough ML, Patel AV, Thun MJ. Association of alcohol intake with pancreatic cancer mortality in never smokers. Arch Intern Med. 2011;171:444-51.

85. Duell EJ. Epidemiology and potential mechanisms of tobacco smoking and heavy alcohol consumption in pancreatic cancer. Mol Carcinog. 2012;51:40-52.

86. World Cancer Research Fund / American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC: AICR; 2007.

87. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS. Physical activity, obesity, height, and the risk of pancreatic cancer. JAMA. 2001;286:921-9.

88. Depeint F, Bruce WR, Shangari N, Mehta R, O'Brien PJ. Mitochondrial function and toxicity: role of B vitamins on the one-carbon transfer pathways. Chem Biol Interact. 2006;163:113-32.

89. Moat SJ, Ashfield-Watt PA, Powers HJ, Newcombe RG, McDowell IF. Effect of riboflavin status on the homocysteine-lowering effect of folate in relation to the MTHFR (C677T) genotype. Clin Chem. 2003;49:295-302.

90. Benavides MA, Bosland MC, da Silva CP, Gomes Sares CT, de Oliveira AM, Kemp R, et al. L-Methionine inhibits growth of human pancreatic cancer cells. Anticancer Drugs. 2014;25:200-3.

91. Miller AL. The methionine-homocysteine cycle and its effects on cognitive diseases. Altern Med Rev. 2003;8:7-19.

92. Aitken SM, Lodha PH, Morneau DJ. The enzymes of the transsulfuration pathways: active-site characterizations. Biochim Biophys Acta. 2011;1814:1511-7.

93. Shane B. Folylpolyglutamate synthesis and role in the regulation of one-carbon metabolism. Vitam Horm. 1989;45:263-335.

94. Townsend JH, Davis SR, Mackey AD, Gregory JF, 3rd. Folate deprivation reduces homocysteine remethylation in a human intestinal epithelial cell culture model: role of serine in one-carbon donation. Am J Physiol Gastrointest Liver Physiol. 2004;286:G588-95.

95. Choi SW, Friso S. Vitamin B6 and Cancer. In: Stanger O, editor. Water Soluble Vitamins: Springer Science+Business Media B.V.; 2012.

96. Eric S. Calhoun SEK. Molecular Genetics of Pancreatic Cancer. In: Lowy AML, S.D.; Philip, P., editor. Pancreatic Cancer2008. p. 750.

97. Sato N, Maitra A, Fukushima N, van Heek NT, Matsubayashi H, Iacobuzio-Donahue CA, et al. Frequent hypomethylation of multiple genes overexpressed in pancreatic ductal adenocarcinoma. Cancer Res. 2003;63:4158-66.

98. Ueki T, Toyota M, Sohn T, Yeo CJ, Issa JP, Hruban RH, et al. Hypermethylation of multiple genes in pancreatic adenocarcinoma. Cancer Res. 2000;60:1835-9.

99. Singh M. Effect of vitamin B6 deficiency on pancreatic acinar cell function. Life Sciences. 1980;26:715-24.

100. Mizumoto K, Tsutsumi M, Denda A, Konishi Y. Rapid production of pancreatic carcinoma by initiation with N-nitroso-bis(2-oxopropyl)amine and repeated augmentation pressure in hamsters. J Natl Cancer Inst. 1988;80:1564-7.

101. Lin HL, An QZ, Wang QZ, Liu CX. Folate intake and pancreatic cancer risk: an overall and dose-response meta-analysis. Public Health. 2013;127:607-13.

102. Larsson SC, Giovannucci E, Wolk A. Methionine and vitamin B6 intake and risk of pancreatic cancer: a prospective study of Swedish women and men. Gastroenterology. 2007;132:113-8.

103. Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J, Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. Am J Epidemiol. 2001;153:680-7.

104. Subar AF, Krebs-Smith SM, Cook A, Kahle LL. Dietary sources of nutrients among US adults, 1989 to 1991. J Am Diet Assoc. 1998;98:537-47.

105. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate OBV, and Choline. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, D.C.: National Academies Press (US); 1998

106. Mackey A, Davis S, Gregory J. Vitamin B6. In: Shils M, Shike M, Ross A, Caballero B, Cousins R, editors. Modern Nutrition in Health and Disease 10th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2005.

107. Paul L, Ueland PM, Selhub J. Mechanistic perspective on the relationship between pyridoxal 5'-phosphate and inflammation. Nutr Rev. 2013;71:239-44.

108. Spinneker A, Sola R, Lemmen V, Castillo MJ, Pietrzik K, Gonzalez-Gross M. Vitamin B6 status, deficiency and its consequences--an overview. Nutr Hosp. 2007;22:7-24.

109. Schernhammer E, Wolpin B, Rifai N, Cochrane B, Manson JA, Ma J, et al. Plasma folate, vitamin B6, vitamin B12, and homocysteine and pancreatic cancer risk in four large cohorts. Cancer Res. 2007;67:5553-60.

110. Chuang SC, Stolzenberg-Solomon R, Ueland PM, Vollset SE, Midttun O, Olsen A, et al. A U-shaped relationship between plasma folate and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. Eur J Cancer. 2011;47:1808-16.

111. Stolzenberg-Solomon RZ, Albanes D, Nieto FJ, Hartman TJ, Tangrea JA, Rautalahti M, et al. Pancreatic cancer risk and nutrition-related methyl-group availability indicators in male smokers. J Natl Cancer Inst. 1999;91:535-41.

112. Vermaak WJ, Ubbink JB, Barnard HC, Potgieter GM, van Jaarsveld H, Groenewald AJ. Vitamin B-6 nutrition status and cigarette smoking. Am J Clin Nutr. 1990;51:1058-61.

113. Bender D. Water-soluble vitamins. . In: Geissler C PH, editor. Modern Nutrition in Health and Disease, 11th ed. Edinburgh: Elsevier; 2005. p. 194-5.

114. Bor MV, Refsum H, Bisp MR, Bleie O, Schneede J, Nordrehaug JE, et al. Plasma vitamin B6 vitamers before and after oral vitamin B6 treatment: a randomized placebo-controlled study. Clin Chem. 2003;49:155-61.

115. Ulvik A, Midttun O, Pedersen ER, Eussen SJ, Nygard O, Ueland PM. Evidence for increased catabolism of vitamin B-6 during systemic inflammation. Am J Clin Nutr. 2014;100:250-5.

116. Sakakeeny L, Roubenoff R, Obin M, Fontes JD, Benjamin EJ, Bujanover Y, et al. Plasma pyridoxal-5-phosphate is inversely associated with systemic markers of inflammation in a population of U.S. adults. J Nutr. 2012;142:1280-5.

117. Shen J, Lai CQ, Mattei J, Ordovas JM, Tucker KL. Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study. Am J Clin Nutr. 2010;91:337-42.

118. Christensen MH, Pedersen EK, Nordbo Y, Varhaug JE, Midttun O, Ueland PM, et al. Vitamin B6 status and interferon-gamma-mediated immune activation in primary hyperparathyroidism. J Intern Med. 2012;272:583-91.

119. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30:1073-81.

120. da Silva VR, Rios-Avila L, Lamers Y, Ralat MA, Midttun O, Quinlivan EP, et al. Metabolite profile analysis reveals functional effects of 28-day vitamin B-6 restriction on one-

carbon metabolism and tryptophan catabolic pathways in healthy men and women. J Nutr. 2013;143:1719-27.

121. Rios-Avila L, Nijhout HF, Reed MC, Sitren HS, Gregory JF, 3rd. A mathematical model of tryptophan metabolism via the kynurenine pathway provides insights into the effects of vitamin B-6 deficiency, tryptophan loading, and induction of tryptophan 2,3-dioxygenase on tryptophan metabolites. J Nutr. 2013;143:1509-19.

122. Rios-Avila L, Coats B, Chi YY, Midttun O, Ueland PM, Stacpoole PW, et al. Metabolite profile analysis reveals association of vitamin B-6 with metabolites related to one-carbon metabolism and tryptophan catabolism but not with biomarkers of inflammation in oral contraceptive users and reveals the effects of oral contraceptives on these processes. J Nutr. 2015;145:87-95.

123. Ulvik A, Theofylaktopoulou D, Midttun O, Nygard O, Eussen SJ, Ueland PM. Substrate product ratios of enzymes in the kynurenine pathway measured in plasma as indicators of functional vitamin B-6 status. Am J Clin Nutr. 2013;98:934-40.

124. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res. 2007;67:9518-27.

125. Huang SC, Wei JC, Wu DJ, Huang YC. Vitamin B(6) supplementation improves pro-inflammatory responses in patients with rheumatoid arthritis. Eur J Clin Nutr. 2010;64:1007-13.

126. Taher YA, Piavaux BJ, Gras R, van Esch BC, Hofman GA, Bloksma N, et al. Indoleamine 2,3-dioxygenase-dependent tryptophan metabolites contribute to tolerance induction during allergen immunotherapy in a mouse model. J Allergy Clin Immunol. 2008;121:983-91 e2.

127. Wormann SM, Diakopoulos KN, Lesina M, Algul H. The immune network in pancreatic cancer development and progression. Oncogene. 2014;33:2956-67.

128. Noakes R. The aryl hydrocarbon receptor: a review of its role in the physiology and pathology of the integument and its relationship to the tryptophan metabolism. Int J Tryptophan Res. 2015;8:7-18.

129. Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, et al. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell. 2011;147:629-40.

130. Monteleone I, Rizzo A, Sarra M, Sica G, Sileri P, Biancone L, et al. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. Gastroenterology. 2011;141:237-48, 48 e1.

131. Wei P, Hu GH, Kang HY, Yao HB, Kou W, Liu H, et al. An aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress the Th17 response in allergic rhinitis patients. Lab Invest. 2014;94:528-35.

132. Koliopanos A, Kleeff J, Xiao Y, Safe S, Zimmermann A, Buchler MW, et al. Increased arylhydrocarbon receptor expression offers a potential therapeutic target for pancreatic cancer. Oncogene. 2002;21:6059-70.

133. Safe S, Lee SO, Jin UH. Role of the aryl hydrocarbon receptor in carcinogenesis and potential as a drug target. Toxicol Sci. 2013;135:1-16.

134. Botwinick IC, Pursell L, Yu G, Cooper T, Mann JJ, Chabot JA. A biological basis for depression in pancreatic cancer. HPB (Oxford). 2014;16:740-3.

135. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004;75:163-89.

136. Detjen KM, Farwig K, Welzel M, Wiedenmann B, Rosewicz S. Interferon gamma inhibits growth of human pancreatic carcinoma cells via caspase-1 dependent induction of apoptosis. Gut. 2001;49:251-62.

137. Younes HM, Amsden BG. Interferon-gamma therapy: evaluation of routes of administration and delivery systems. J Pharm Sci. 2002;91:2-17.

138. Zuo H, Tell GS, Vollset SE, Ueland PM, Nygard O, Midttun O, et al. Interferon-gamma-induced inflammatory markers and the risk of cancer: the Hordaland Health Study. Cancer. 2014;120:3370-7.

139. Ries LAG YJ, Keel GE, Eisner MP, Lin YD, Horner M-J (editors),. SEER Survival Monograph: Cancer Survival Among Adults: U.S. SEER Program, 1988-2001, Patient and Tumor Characteristics. . Bethesda, MD: National Cancer Institute, SEER Program, NIH Pub. No. 07-6215; 2007.

140. Jacobs EJ, Chanock SJ, Fuchs CS, Lacroix A, McWilliams RR, Steplowski E, et al. Family history of cancer and risk of pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). Int J Cancer. 2010;127:1421-8.

141. Larsson SC, Wolk A. Red and processed meat consumption and risk of pancreatic cancer: meta-analysis of prospective studies. Br J Cancer. 2012;106:603-7.

142. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Fuchs CS. Dietary meat, dairy products, fat, and cholesterol and pancreatic cancer risk in a prospective study. Am J Epidemiol. 2003;157:1115-25.

143. Lucenteforte E, La Vecchia C, Silverman D, Petersen GM, Bracci PM, Ji BT, et al. Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Ann Oncol. 2012;23:374-82.

144. Chan JM, Wang F, Holly EA. Vegetable and fruit intake and pancreatic cancer in a population-based case-control study in the San Francisco bay area. Cancer Epidemiol Biomarkers Prev. 2005;14:2093-7.

145. American Cancer Society. Cancer Facts & Figures 2014. Atlanta: American Cancer Society; 2014.

146. Iodice S, Gandini S, Maisonneuve P, Lowenfels AB. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. Langenbecks Arch Surg. 2008;393:535-45.

147. Aune D, Greenwood DC, Chan DS, Vieira R, Vieira AR, Navarro Rosenblatt DA, et al. Body mass index, abdominal fatness and pancreatic cancer risk: a systematic review and non-linear dose-response meta-analysis of prospective studies. Ann Oncol. 2012;23:843-52.

148. Selhub J. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. J Nutr Health Aging. 2002;6:39-42.

149. Lomberk G, Mathison AJ, Grzenda A, Urrutia R. The sunset of somatic genetics and the dawn of epigenetics: a new frontier in pancreatic cancer research. Curr Opin Gastroenterol. 2008;24:597-602.

150. Tan AC, Jimeno A, Lin SH, Wheelhouse J, Chan F, Solomon A, et al. Characterizing DNA methylation patterns in pancreatic cancer genome. Mol Oncol. 2009;3:425-38.

151. Sato N, Fukushima N, Maitra A, Matsubayashi H, Yeo CJ, Cameron JL, et al. Discovery of novel targets for aberrant methylation in pancreatic carcinoma using high-throughput microarrays. Cancer Res. 2003;63:3735-42.

152. Choi SW, Friso S. Vitamins B6 and cancer. Subcell Biochem. 2012;56:247-64.

153. Jimenez-Chillaron JC, Diaz R, Martinez D, Pentinat T, Ramon-Krauel M, Ribo S, et al. The role of nutrition on epigenetic modifications and their implications on health. Biochimie. 2012;94:2242-63.

154. Pogribny IP, Tryndyak VP, Bagnyukova TV, Melnyk S, Montgomery B, Ross SA, et al. Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. J Hepatol. 2009;51:176-86.

155. Pogribny IP, Karpf AR, James SR, Melnyk S, Han T, Tryndyak VP. Epigenetic alterations in the brains of Fisher 344 rats induced by long-term administration of folate/methyl-deficient diet. Brain Res. 2008;1237:25-34.

156. Andry CD, Kupchik HZ, Rogers AE. L-azaserine induced preneoplasia in the rat pancreas. A morphometric study of dietary manipulation (lipotrope deficiency) and ultrastructural differentiation. Toxicol Pathol. 1990;18:10-7.

157. Longnecker DS, Chandar N, Sheahan DG, Janosky JE, Lombardi B. Preneoplastic and neoplastic lesions in the pancreas of rats fed choline-devoid or choline-supplemented diets. Toxicol Pathol. 1991;19:59-65.

158. Gong Z, Holly EA, Bracci PM. Intake of folate, vitamins B6, B12 and methionine and risk of pancreatic cancer in a large population-based case-control study. Cancer Causes Control. 2009;20:1317-25.

159. Yuan JM, Stram DO, Arakawa K, Lee HP, Yu MC. Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev. 2003;12:890-8.

160. Hankin JH, Stram DO, Arakawa K, Park S, Low SH, Lee HP, et al. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. Nutr Cancer. 2001;39:187-95.

161. US Department of Agriculture. USDA Nutrient Data Base for Individual Food Intake Surveys and Data Sets Used to Create It, release 7. Springfield, VA: National Technical Information Service; 1994.

162. US Department of Agriculture. Composition of Foods: Raw, Processed, Prepared: Nutrient Data Base for Standard Reference, release 10. Riverdale, MD: Nutrient Data Laboratory, Agricultural Research Service; 1993.

163. Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine. Chinese Food Composition Tables. In: Press NC, editor. Beijing, China1991.

164. Siong T, Noor, MI, Azudin, MN, and Idris, K. Nutrient Composition of Malaysian Foods. Malaysia: ASEAN Food Habits Project, Food Habits Research and Development; 1998.

165. Department of Health, Executive Yuan, Republic of China. Food Composition Table in Taiwan Area. Republic of China: Department of Health; 1994.

166. Willett W. Nutritional Epidemiology. 2nd ed: Oxford University Press; 1998.

167. Howe JC, Williams JR, Holden JM, Zeisel SH, Mar MH. USDA Database for the Choline Content of Common Foods, Release One. 2004.

168. Patterson KY, Bhagwat SA, Williams JR, Howe JC, Holden JM, Zeisel SH, et al. USDA Database for the Choline Content of Common Foods, Release Two. 2008.

169. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 19. 2006.

170. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 20. 2007.

171. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 21. 2008.

172. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc. 1988;88:1268-71.

173. Parkin DM, Whelan SI, Ferlay J, Teppo L, Thomas D. Cancer Incidence in Five Continents. Volume VII2002.

174. Cox DR. Regression Models and Life-Tables. Journal of the Royal Statistical Society Series B (Methodological). 1972;34:187-220.

175. Untawale S, Odegaard AO, Koh WP, Jin AZ, Yuan JM, Anderson KE. Body mass index and risk of pancreatic cancer in a Chinese population. PLoS One. 2014;9:e85149.

176. W. H. O. Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363:157-63.

177. Olsen A, Halkjaer J, van Gils CH, Buijsse B, Verhagen H, Jenab M, et al. Dietary intake of the water-soluble vitamins B1, B2, B6, B12 and C in 10 countries in the European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr. 2009;63 Suppl 4:S122-49.

178. Cotton PA, Subar AF, Friday JE, Cook A. Dietary sources of nutrients among US adults, 1994 to 1996. J Am Diet Assoc. 2004;104:921-30.

179. World Cancer Research Fund, American Institute for Cancer Research. Food, nutrition, phyiscal activity, and the prevention of cancer: A global persepctive. Washington, DC: AICR; 2007.

180. Inoue-Choi M, Nelson HH, Robien K, Arning E, Bottiglieri T, Koh WP, et al. One-carbon metabolism nutrient status and plasma S-adenosylmethionine concentrations in middle-aged and older Chinese in Singapore. Int J Mol Epidemiol Genet. 2012;3:160-73.

181. Perry C, Yu S, Chen J, Matharu KS, Stover PJ. Effect of vitamin B6 availability on serine hydroxymethyltransferase in MCF-7 cells. Arch Biochem Biophys. 2007;462:21-7.

182. Stabler SP, Sampson DA, Wang LP, Allen RH. Elevations of serum cystathionine and total homocysteine in pyridoxine-, folate-, and cobalamin-deficient rats. The Journal of Nutritional Biochemistry. 1997;8:279-89.

183. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res. 2001;475:7-20.

184. Huang YC, Chen W, Evans MA, Mitchell ME, Shultz TD. Vitamin B-6 requirement and status assessment of young women fed a high-protein diet with various levels of vitamin B-6. Am J Clin Nutr. 1998;67:208-20.

185. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. Nature. 1998;395:89-93.

186. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, et al. Induction of tumors in mice by genomic hypomethylation. Science. 2003;300:489-92.

187. Timp W, Bravo HC, McDonald OG, Goggins M, Umbricht C, Zeiger M, et al. Large hypomethylated blocks as a universal defining epigenetic alteration in human solid tumors. Genome Med. 2014;6:61.

188. Weisenberger DJ, Campan M, Long TI, Kim M, Woods C, Fiala E, et al. Analysis of repetitive element DNA methylation by MethyLight. Nucleic Acids Res. 2005;33:6823-36.

189. Woo HD, Kim J. Global DNA hypomethylation in peripheral blood leukocytes as a biomarker for cancer risk: a meta-analysis. PLoS One. 2012;7:e34615.

190. Inoue-Choi M, Nelson HH, Robien K, Arning E, Bottiglieri T, Koh WP, et al. Plasma S-adenosylmethionine, DNMT polymorphisms, and peripheral blood LINE-1 methylation among healthy Chinese adults in Singapore. BMC Cancer. 2013;13:389.

191. Dubick MA, Gretz D, Majumdar APN. Overt vitamin B-6 deficiency affects rat pancreatic digestive enzyme and glutathione reductase activities. J Nutr. 1995;125:20-5.

192. Longnecker DS. Abnormal methyl metabolism in pancreatic toxicity and diabetes. J Nutr. 2002;132:2373S-6S.

193. Ida S, Ohmuraya M, Hirota M, Ozaki N, Hiramatsu S, Uehara H, et al. Chronic pancreatitis in mice by treatment with choline-deficient ethionine-supplemented diet. Exp Anim. 2010;59:421-9.

194. Mizumoto K, Tsutsumi M, Kitazawa S, Denda A, Konishi Y. Usefulness of rapid production model for pancreatic carcinoma in male hamsters. Cancer Lett. 1990;49:211-5.

195. Skinner HG, Michaud DS, Giovannucci EL, Rimm EB, Stampfer MJ, Willett WC, et al. A prospective study of folate intake and the risk of pancreatic cancer in men and women. Am J Epidemiol. 2004;160:248-58.

196. Fischer LM, daCosta KA, Kwock L, Stewart PW, Lu TS, Stabler SP, et al. Sex and menopausal status influence human dietary requirements for the nutrient choline. Am J Clin Nutr. 2007;85:1275-85.

197. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359-86.

198. Jansen RJ, Robinson DP, Stolzenberg-Solomon RZ, Bamlet WR, de Andrade M, Oberg AL, et al. Fruit and vegetable consumption is inversely associated with having pancreatic cancer. Cancer Causes Control. 2011;22:1613-25.

199. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28 (Slightly revised). Version Current: May 2016. Internet: http://www.ars.usda.gov/sp2UserFiles/Place/80400525/Data/SR/SR28/sr28_doc.pdf.

200. Huang JY, Butler LM, Wang R, Jin A, Koh WP, Yuan JM. Dietary intake of one-carbon metabolism-related nutrients and pancreatic cancer risk: The Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev. 2016;25:417-24.

201. Albersen M, Bosma M, Luykx JJ, Jans JJ, Bakker SC, Strengman E, et al. Vitamin B-6 vitamers in human plasma and cerebrospinal fluid. Am J Clin Nutr. 2014;100:587-92.

202. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF. Direct and functional biomarkers of vitamin B6 status. Annu Rev Nutr. 2015;35:33-70.

203. Greer JB, Whitcomb DC. Inflammation and pancreatic cancer: an evidence-based review. Curr Opin Pharmacol. 2009;9:411-8.

204. Yuan JM, Ross RK, Wang XL, Gao YT, Henderson BE, Yu MC. Morbidity and mortality in relation to cigarette smoking in Shanghai, China. A prospective male cohort study. JAMA. 1996;275:1646-50.

205. Odegaard AO, Pereira MA, Koh WP, Arakawa K, Lee HP, Yu MC. Coffee, tea, and incident type 2 diabetes: the Singapore Chinese Health Study. Am J Clin Nutr. 2008;88:979-85.

206. Odegaard AO, Koh WP, Arakawa K, Yu MC, Pereira MA. Soft drink and juice consumption and risk of physician-diagnosed incident type 2 diabetes: the Singapore Chinese Health Study. Am J Epidemiol. 2010;171:701-8.

207. Yuan JM, Koh WP, Murphy SE, Fan Y, Wang R, Carmella SG, et al. Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. Cancer Res. 2009;69:2990-5.

208. Midttun O, Kvalheim G, Ueland PM. High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. Anal Bioanal Chem. 2013;405:2009-17.

209. Breslow N, Day N. Statistical methods in cancer research, vol.1: The analysis of case–control studies. Lyon: IARC: IARC Scientific Pub No. 32.; 1980.

210. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate OBV, and Choline. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington (DC)1998.

211. Altman DG, Bland JM. Interaction revisited: the difference between two estimates. BMJ. 2003;326:219.

212. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150:604-12.

213. Shin-Buehring Y, Rasshofer R, Endres W. A new enzymatic method for pyridoxal 5'-phosphate determination. J Inherit Metab Disorders. 1981;4:123–24.

214. Kannan K, Jain SK. Effect of vitamin B6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H2O2-treated U937 monocytes. Free Radic Biol Med. 2004;36:423-8.

215. Kuwahara K, Nanri A, Pham NM, Kurotani K, Kume A, Sato M, et al. Serum vitamin B6, folate, and homocysteine concentrations and oxidative DNA damage in Japanese men and women. Nutrition. 2013;29:1219-23.

216. Marzio A, Merigliano C, Gatti M, Verni F. Sugar and chromosome stability: clastogenic effects of sugars in vitamin B6-deficient cells. PLoS Genet. 2014;10:e1004199.

217. Jiao L, Weinstein SJ, Albanes D, Taylor PR, Graubard BI, Virtamo J, et al. Evidence that serum levels of the soluble receptor for advanced glycation end products are inversely associated with pancreatic cancer risk: a prospective study. Cancer Res. 2011;71:3582-9.

218. Jiao L, Taylor PR, Weinstein SJ, Graubard BI, Virtamo J, Albanes D, et al. Advanced glycation end products, soluble receptor for advanced glycation end products, and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2011;20:1430-8.

219. Leklem JE. Vitamin B-6: a status report. J Nutr. 1990;120 Suppl 11:1503-7.

220. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Altekruse SF, et al. SEER Cancer Statistics Review, 1975-2013, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2013/, based on November 2015 SEER data submission, posted to the SEER web site, April 2016.

221. Blackford A, Parmigiani G, Kensler TW, Wolfgang C, Jones S, Zhang X, et al. Genetic mutations associated with cigarette smoking in pancreatic cancer. Cancer Res. 2009;69:3681-8.

222. Stolzenberg-Solomon RZ, Schairer C, Moore S, Hollenbeck A, Silverman DT. Lifetime adiposity and risk of pancreatic cancer in the NIH-AARP Diet and Health Study cohort. Am J Clin Nutr. 2013;98:1057-65.

223. Chiang EP, Smith DE, Selhub J, Dallal G, Wang YC, Roubenoff R. Inflammation causes tissue-specific depletion of vitamin B6. Arthritis Res Ther. 2005;7:R1254-62.

224. Kolodziej LR, Paleolog EM, Williams RO. Kynurenine metabolism in health and disease. Amino Acids. 2011;41:1173-83.

225. Pingle SK, Tumane RG, Jawade AA. Neopterin: Biomarker of cell-mediated immunity and potent usage as biomarker in silicosis and other occupational diseases. Indian J Occup Environ Med. 2008;12:107-11.

226. Chuang SC, Fanidi A, Ueland PM, Relton C, Midttun O, Vollset SE, et al. Circulating biomarkers of tryptophan and the kynurenine pathway and lung cancer risk. Cancer Epidemiol Biomarkers Prev. 2014;23:461-8.

227. Le Floc'h N, Otten W, Merlot E. Tryptophan metabolism, from nutrition to potential therapeutic applications. Amino Acids. 2011;41:1195-205.

228. Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature. 2011;478:197-203.

229. Lowe MM, Mold JE, Kanwar B, Huang Y, Louie A, Pollastri MP, et al. Identification of cinnabarinic acid as a novel endogenous aryl hydrocarbon receptor ligand that drives IL-22 production. PLoS One. 2014;9:e87877.

230. Ogasawara N, Hagino Y, Kotake Y. Kynurenine-transaminase, kynureninase and the increase of xanthurenic acid excretion. J Biochem. 1962;52:162-6.

231. Desvignes L, Ernst JD. Interferon-gamma-responsive nonhematopoietic cells regulate the immune response to Mycobacterium tuberculosis. Immunity. 2009;31:974-85.

232. McAllister F, Bailey JM, Alsina J, Nirschl CJ, Sharma R, Fan H, et al. Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia. Cancer Cell. 2014;25:621-37.

233. Theofylaktopoulou D, Midttun O, Ulvik A, Ueland PM, Tell GS, Vollset SE, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study. Clin Exp Immunol. 2013;173:121-30.

234. Niculescu MD, Zeisel SH. Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. J Nutr. 2002;132:2333S-5S.

235. Huang JY, Butler LM, Wang R, Jin A, Koh WP, Yuan JM. Dietary Intake of One-Carbon Metabolism-Related Nutrients and Pancreatic Cancer Risk: The Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev. 2015.

236. Galluzzi L, Vacchelli E, Michels J, Garcia P, Kepp O, Senovilla L, et al. Effects of vitamin B6 metabolism on oncogenesis, tumor progression and therapeutic responses. Oncogene. 2013;32:4995-5004.

237. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2009;23:1371-9.

238. American Cancer Society. Cancer Facts & Figures 2015. Atlanta: American Cancer Society; 2015.

239. Bruzzi P, Green SB, Byar DP, Brinton LA, Schairer C. Estimating the population attributable risk for multiple risk factors using case-control data. Am J Epidemiol. 1985;122:904-14.

240. Johansson M, Relton C, Ueland PM, Vollset SE, Midttun O, Nygard O, et al. Serum B vitamin levels and risk of lung cancer. JAMA. 2010;303:2377-85.

241. Zhang XH, Ma J, Smith-Warner SA, Lee JE, Giovannucci E. Vitamin B6 and colorectal cancer: current evidence and future directions. World J Gastroenterol. 2013;19:1005-10.

242. de Lourdes Samaniego-Vaesken M, Alonso-Aperte E, Varela-Moreiras G. Vitamin food fortification today. Food Nutr Res. 2012;56.

243. Iacobuzio-Donahue CA. Genetic evolution of pancreatic cancer: lessons learnt from the pancreatic cancer genome sequencing project. Gut. 2012;61:1085-94.

244. Midttun O, Townsend MK, Nygard O, Tworoger SS, Brennan P, Johansson M, et al. Most blood biomarkers related to vitamin status, one-carbon metabolism, and the kynurenine pathway show adequate preanalytical stability and within-person reproducibility to allow assessment of exposure or nutritional status in healthy women and cardiovascular patients. J Nutr. 2014;144:784-90.

245. Jacob RA, Jenden DJ, Allman-Farinelli MA, Swendseid ME. Folate nutriture alters choline status of women and men fed low choline diets. J Nutr. 1999;129:712-7.

246. van Wijk N, Watkins CJ, Bohlke M, Maher TJ, Hageman RJ, Kamphuis PJ, et al. Plasma choline concentration varies with different dietary levels of vitamins B6, B12 and folic acid in rats maintained on choline-adequate diets. Br J Nutr. 2012;107:1408-12.

247. Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat Rev Cancer. 2013;13:572-83.

248. Glunde K, Bhujwalla ZM, Ronen SM. Choline metabolism in malignant transformation. Nat Rev Cancer. 2011;11:835-48.

249. Balendiran GK, Dabur R, Fraser D. The role of glutathione in cancer. Cell Biochem Funct. 2004;22:343-52.