SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
NUTRITION
RISK FACTORS

Received
12 August 2014

Accepted
16 December 2014

Published
4 February 2015

Correspondence and requests for materials should be addressed to S.Z. (shuguang0888@sohu.com or shuguang828@163.com)

Association between cholesterol intake and pancreatic cancer risk: Evidence from a meta-analysis

Hongqiang Chen, Shiyong Qin, Minghai Wang, Tao Zhang & Shuguang Zhang

Department of General Surgery, Shandong Provincial Qianfoshan Hospital, Shandong University, Jinan, Shandong 250014, P.R. China.

Quantification of the association between the intake of cholesterol and risk of pancreatic cancer is still conflicting. We therefore conducted a meta-analysis to summarize the evidence from epidemiological studies of cholesterol intake and the risk of pancreatic cancer. Pertinent studies were delivered by PubMed and Web of Knowledge issued through April of 2014. A random effects model was used to process the data for analysis. Sensitivity analysis and publication bias were conducted. Dose-response relationship was assessed by restricted cubic spline and variance-weighted least squares regression analysis. With 4513 pancreatic cases exemplified, 16 articles were applied in the meta-analysis. Pooled results suggest that cholesterol intake level was significantly associated with the risk of pancreatic cancer [summary relative risk (RR) =1.371, 95%CI=1.155–1.627, I^2=58.2%], especially in America [summary RR =1.302, 95%CI=1.090–1.556]. A linear dose-response relation was attested that the risk of pancreatic cancer rises by 8% with 100 mg/day of cholesterol intake. [summary RR = 1.08, 95% CI = 1.04–1.13]. In conclusion, our analysis suggests that a high intake of cholesterol might increase the risk of pancreatic cancer, especially in America.

Pancreatic cancer is one of the most dismal malignancies. Lacking highly sensitive and specific test methods and early symptoms, early diagnosis and treatment are rarely satisfactory, much less than discovery. Pancreatic cancer as an aggressive malignancy takes the eighth place in cancer-related mortality worldwide, the estimated deaths from pancreatic cancer are 39,590 in United States[1]. However, the only option for cure is surgery and only 20% patients have such chance due to late detection and diagnosis[2]. Thus, primary prevention is a priority. The recent genome-wide association studies (GWAS) showed that pancreatic cancer is associated with genetic factors[3,4]. Furthermore, several other modifiable risk factors have been confirmed the risk of pancreatic cancer, including cigarette smoking, diabetes, alcohol intake, obesity, chronic pancreatitis and diet[5–9].

It has been hypothesized that higher intake of cholesterol may be associated with an elevated risk of pancreatic cancer[10]. Up to date, a number of epidemiologic studies have been published to explore the relationship between cholesterol intake and pancreatic cancer risk. However, the results are not consistent. Therefore, we conducted a meta-analysis to assess the pancreatic cancer risk for the highest vs. lowest categories of cholesterol intake and assess the dose-response association of pancreatic cancer for every 100 mg/day increment in cholesterol intake. Furthermore, we also assess the heterogeneity among studies and publication bias.

Methods

Search Strategy. Studies were identified by using a literature search of PubMed and Web of Knowledge through April of 2014 and by hand-searching the reference lists of the retrieved articles. The following search terms were used: 'pancreatic cancer' or 'pancreatic carcinoma' combined with 'nutrition', 'diet', 'lifestyle' or 'cholesterol'. Two investigators searched articles and reviewed all the retrieved studies independently.

Study Selection. For inclusion, studies had to fulfill the following criteria: (1) prospective or case-control study design; (2) cholesterol intake was the independent variable of interest; (3) the dependent variable of interest was pancreatic cancer; (4) relative risk (RR) or odds ratio (OR) with 95% confidence interval (CI) was provided; and (5) for dose-response analysis, the intake of cholesterol for each response category must also have been provided (or data available to calculate them).

Data extraction. Two researchers independently extracted the following data from the included studies: the first author's last name, year of publication, geographic locations, study design, sample source, the age range of study participants, duration of follow-up, the number of

cases and participants (person-years), and RR (95%CI) for each category of cholesterol. From each study, we extracted the RR that reflected the greatest degree of control for potential confounders.

Statistical analysis. We carried out a random-effect dose–response meta-analysis with the method proposed by Greenland and Longnecker[11] and Orsini et al.[12], which takes into account the correlation between the log RR estimates across categories of cholesterol intake. We also explored the possibility of nonlinear relationships by modeling cholesterol intake by using restricted cubic splines with three knots (i.e. two spline transformations) at fixed percentiles (25%, 50% and 75%) of cholesterol intake distribution. A P-value for nonlinearity was calculated by testing against the null hypothesis that the coefficient of the second spline transformation was equal to zero[13]. The preconditions for the methods are that the distribution of cases and person-years or noncases and the RR with the variance estimates for at least three quantitative exposure categories are known. When this information was not available, we estimated the slopes (linear trends) by using variance-weighted least squares regression analysis[14,15]. The median cholesterol intake for each specific category was assigned to each corresponding log RR estimate. If the median intake was not reported in the article, we used the midpoint between the upper and lower boundary. If the lowest category was open-ended, its lower boundary was set to zero. If the upper boundary of the highest category was left unspecified, we assumed the category to be of the same amplitude as the preceding one. Statistical heterogeneity across studies was assessed using the Q and I^2 statistics[16]. An I^2 statistic $<30\%$ indicated no or marginal between-study heterogeneity, 30%–75% considerable moderate heterogeneity and $>75\%$ considerable heterogeneity. Meta-regression with restricted maximum likelihood estimation was performed to assess the potentially important covariates that might exert substantial impact on between-study heterogeneity[17]. A study of influence analysis[18] was conducted to describe how robust the pooled estimator was to removal of individual studies. Publication bias was evaluated by means of Egger's regression test[19].

All statistical analyses were conducted with STATA version 11.0 (StataCorp LP, College Station, Texas, USA). Two-tailed $P \leq 0.05$ was accepted as statistically significant.

Results

Search results and study characteristics. The research strategy helped the researchers to collect 253 articles from PubMed and 358 from the Web of Knowledge, with 36 articles reviewed fully after reading the titles and the abstracts. By studying reference lists, we identified 3 additional articles. Twenty-three of these 39 articles were subsequently excluded from the meta-analysis for various reasons. In total, 16 articles[20–35] (4 cohort studies and 12 case-control studies) involving 4513 pancreatic cancer cases were used in this meta-analysis. The detailed steps of our literature search are shown in Figure 1. The characteristics of these studies are presented in Table 1. Four studies were conducted in the United States, 3 in the Canada, 2 in the Netherlands, 1 in the Australia, 1 in the Poland, 1 in the Greece, 1 in the Finland, 1 in the Japan and 1 in the Italy.

High versus low analyses. Data from 16 articles including 4513 pancreatic cancer cases were used in this meta-analysis. Six of the studies included in our analysis report that cholesterol intake could increase the risk of pancreatic cancer, while no significant association was reported in 10 studies. Our pooled results suggested that the highest cholesterol intake level compared to the lowest level was significantly associated with the risk of pancreatic cancer [summary RR=1.371, 95%CI=1.155–1.627, I2=58.2%] (Figure 2).

When the studies were stratified by design, the association was also found in the case-control studies [summary RR=1.577, 95%CI=1.298–1.915] but not in the cohort studies. In subgroup analyses for geographic locations, highest cholesterol intake level versus lowest level was significantly associated with the risk of pancreatic cancer in America [summary RR=1.302, 95%CI=1.090–1.556], but not in the Europe or others. The details results are summarized in Table 2.

Dose-response analysis. For dose-response analysis, data from 5 studies[29,30,32,33,35] comprising 2163 pancreatic cancer cases were used for cholesterol intake and pancreatic cancer risk. We found no evidence of statistically significant departure from linearity (P for nonlinearity = 0.24). Our dose-response analysis indicates that an increase in cholesterol intake of 100 mg/day is statistically significantly associated with an 8% increase in the risk of developing pancreatic cancer (summary RR=1.08, 95%CI=1.04–1.13) (Figure 3).

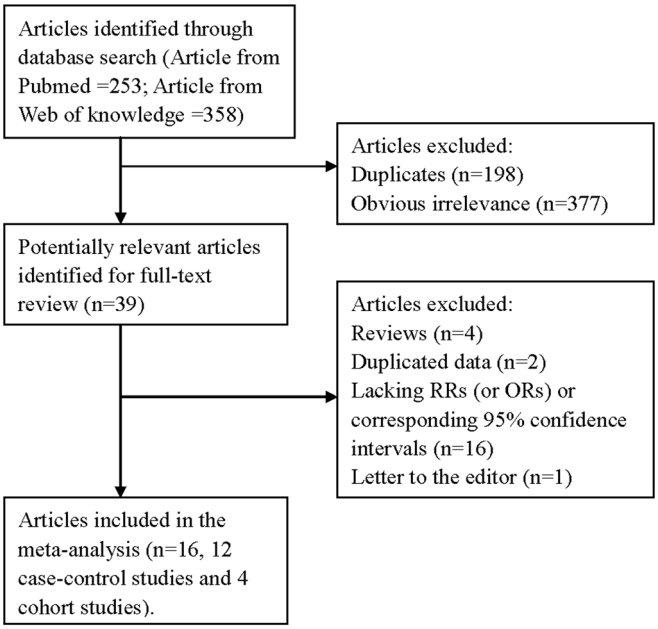

Figure 1 | The flow diagram of screened, excluded, and analyzed publications.

Sources of heterogeneity and meta-regression. As shown in Figure 2, evidence of heterogeneity ($I^2=58.2\%$, $P_{heterogeneity}=0.002$) was found in the pooled results. In order to explore the moderate to high between-study heterogeneity founded in several analysis, univariate meta-regression with the covariates of publication year, location where the study was conducted, study design (case-control or cohort), number of cases and source of controls was performed. No significant findings were found in the above-mentioned analysis.

Influence analysis and publication bias. Influence analysis shows that no individual study exerted excessive influence on the association of cholesterol intake and pancreatic cancer risk. Egger's test ($P=0.164$) showed no evidence of significant publication bias related to the association between cholesterol intake and pancreatic cancer risk.

Discussion

Finding from this meta-analysis suggests that the higher intake of cholesterol could increase the risk of pancreatic cancer. The associations were also found in subgroups of America and case-control studies of cholesterol intake and pancreatic cancer risk.

Several mechanisms have been proposed to explain the possible role of cholesterol in cancer development. Alterations in lipid and apolipoprotein levels could contribute to cellular inflammation[36]. Decreased levels of HDL-C and increased low-density lipoprotein cholesterol (LDL-C) and total cholesterol levels have been related to increased levels of proinflammatory cytokines, including tumor necrosis factor-a and interleukin-6[37]. The Framingham Offspring Cohort study suggests that elevated serum iron levels coupled with either high very low density lipoprotein cholesterol or low HDL-C appeared to interact to increase cancer risk[38]. Another cohort study indicated that independent elevations of either iron or total cholesterol were not significantly related to the development of cancer, but a combination of iron and total cholesterol above the 75th percentile was associated with significant increases in the risk of all cancers and supported the theory that the iron-induced oxidation of serum lipids is important in the pathogenesis of cancer[39].

As a meta-analysis of published studies, our findings showed some advantages. First, we report here the first comprehensive dose-response meta-analysis of cholesterol intake and pancreatic cancer

Table 1 | Characteristics of studies on cholesterol intake and pancreatic cancer risk

Study, Year (Ref.)	Country	Study design	Participants (cases)	Age (years)	RR (95%CI) for highest versus lowest category	Adjustment for covariates
Howe et al. 1990 [20]	Canada	Case-control	754 (249)	35–79	0.95(0.51–1.75)	Adjust for caloric and fibre intake, lifetime cigarette consumption.
Baghurst et al. 1991 [21]	Australia	Case-control	357 (104)	<50–≥80	3.19(1.58–6.47)	Adjust for age; pack-years of smoking, tobacco consumption and vice versa.
Bueno de Mesquita et al. 1991 [22]	Netherlands	Case-control	644 (164)	35–79	1.33(0.72–2.45)	Adjust for age, sex, response status, total smoking and dietary intake of energy.
Zatonski et al. 1991 [23]	Poland	Case-control	305 (110)	62.2	4.31(1.60–11.59)	Adjust for cigarette lifetime consumption and calories.
Olsen et al. 1991 [24]	United States	Case-control	432 (212)	40–84	1.5(0.8–2.6)	Adjusted for total energy, age, cigarette usage, alcohol consumption, respondent-reported history of diabetes mellitus, and educational level.
Howe et al. 1992 [25]	Europe	Case-control	2471 (802)	28–87	2.13(1.42–3.20)	Adjusted for age, sex, nutrient variables (categorical), and lifetime cigarette consumption (continuous).
Kalapothaki et al. 1993 [26]	Greece	Case-control	362 (181)	*Na*	1.19(0.96–1.47)	Adjust for age, gender, hospital, past residence, years of schooling, cigarette smoking, diabetes mellitus and energy intake.
Ghadirian et al. 1995 [27]	Canada	Case-control	418 (179)	35–79	2.24(0.83–6.05)	Adjust for age, sex, lifetime cigarette consumption, response status, and total energy intake.
Stolzenberg-Solomon et al. 2002 [28]	Finland	Cohort	27111 (163)	50–69	0.92(0.53–1.59)	Adjust for by the residual method and for age and years of smoking, energy-adjusted folate intake and energy-adjusted saturated fat intake.
Michaud et al. 2003 [29]	United States	Cohort	88802 (178)	30–55	1.11(0.67–1.83)	Adjust for age, pack-years of smoking, body mass index, history of diabetes mellitus, caloric intake, height, physical activity, menopausal status, and glycemic load intake.
Nothlings et al. 2005 [30]	United States	Cohort	190545 (482)	45–75	1.09(0.89–1.32)	Adjust for age at cohort entry, ethnicity, history of diabetes mellitus, and familial history of pancreatic cancer, smoking status, and energy intake.
Lin et al. 2005 [31]	Japan	Case-control	327 (109)	40–79	2.06(1.11–3.85)	Adjust for age, pack-years of smoking and energy intake.
Chan et al. 2007 [32]	United States	Case-control	2233 (532)	21–85	1.5(1.1–2.0)	Adjust for age, sex using energy-adjusted residual model, body mass index, race, education, smoking and history of diabetes using energy-adjusted residual model.
Heinen et al. 2009 [33]	Netherlands	Cohort	120852 (350)	55–69	0.78(0.52–1.18)	Adjust for gender, age, energy, smoking, alcohol, history of diabetes mellitus, history of hypertension, body mass index, vegetables and fruit.
Lucenteforte et al. 2010 [34]	Italy	Case-control	978 (326)	34–80	1.10(0.68–1.77)	Adjust for age, sex, centre year of interview, education, tobacco smoking, history of diabetes and total energy intake.
Hu et al. 2012 [35]	Canada	Case-control	5667 (628)	20–76	1.57(1.09–2.26)	Adjust for sex, age, province, education, body mass index, alcohol drinking, pack-year smoking, total of vegetable and fruit intake, saturated fat and total energy intake.

Abbreviations: Ref. =references; CI=confidence interval; RR=relative risk; Na= not available.

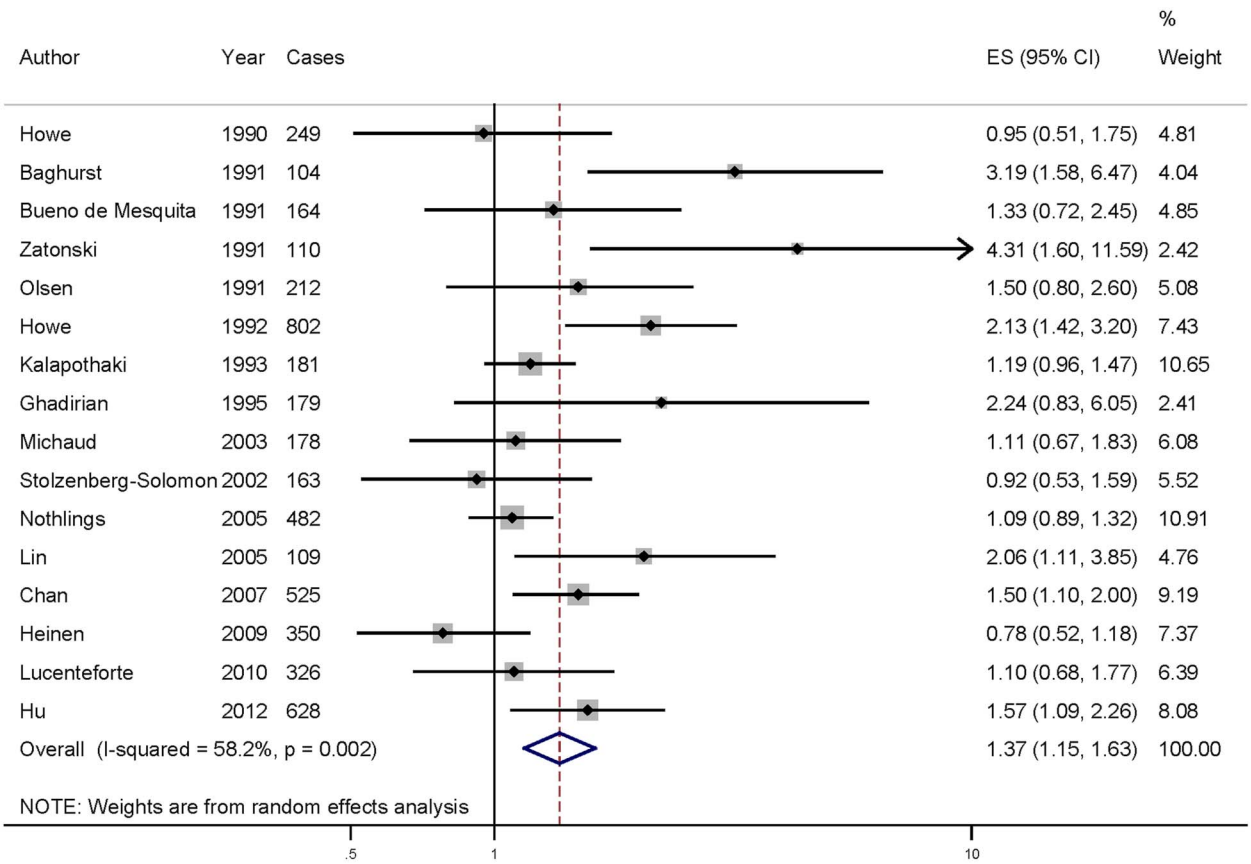

Figure 2 | **The forest plot between highest versus lowest categories of cholesterol intake and pancreatic cancer risk.**

risk based on high versus low analysis and dose-response meta-analysis. Second, our study employed a large number of participants, allowing a much greater possibility of reaching reasonable conclusions between cholesterol intake and pancreatic cancer risk. Third, no significant publication bias was found, indicating that our results are stable. However, there were some limitations in this meta-analysis. First of all, a meta-analysis of observational studies is susceptible to potential bias inherent in the original studies, especially for case-control studies. Overstated association may be expected from the case-control studies because of recall or selection bias, and early symptoms in patients may have resulted in a change in dietary habits. In our meta-analysis, the significant association was found in case-control studies, but not in the cohort studies, while only 4 studies included were prospective design. More studies with prospective design are recommended in the future studies. Therefore, this meta-analysis only discovers "an association" between the cause "cholesterol" and the effect "pancreatic cancer". The increasing of cholesterol intake may result in the risk of pancreatic cancer. Second, measurement errors tend to influence the assessment of dietary intake, which can lead to overestimation of the range of intake and underestimation of the magnitude of the relationship between dietary intake and cancer risk[40,41]. Third, for the subgroups of geographic locations, the association was only significant in the America, but not in the Europe. And only one study comes from Japan and one from Austrilia. Due to the limitation, the results are applicable to the America, but not referential populations elsewhere. More studies conducted in other countries are required to investigate the association between cholesterol intake and pancreatic cancer risk. Fourth, between-study heterogeneity was found in some analysis in this meta-analysis, but not fully explained by the subgroup analysis and meta-regression. However, other genetic and environment variables, as well as their possible interaction may be potential contributors to this disease-effect unconformity.

In summary, results from this meta-analysis suggest that a high intake of cholesterol might increase the risk of pancreatic cancer, especially in America. Dose-response analysis indicates that the risk of pancreatic cancer estimatedly increases by 8% with every 100 mg/day intake of cholesterol.

Table 2 | Summary risk estimates of the association between cholesterol intake and pancreatic cancer risk

Subgroups	No. (cases)	No. studies	Risk estimate (95% CI)	Heterogeneity test I^2 (%)	P-value
All studies	4513	16	1.371(1.155–1.627)	58.2	0.002
Study design					
Prospective	1173	4	1.023(0.871–1.200)	0.0	0.508
Case-control	3340	12	1.577(1.298–1.915)	49.3	0.022
Geographic locations					
America	2453	7	1.302(1.090–1.556)	26.5	0.217
Europe	2096	7	1.291(0.949–1.756)	69.0	0.004

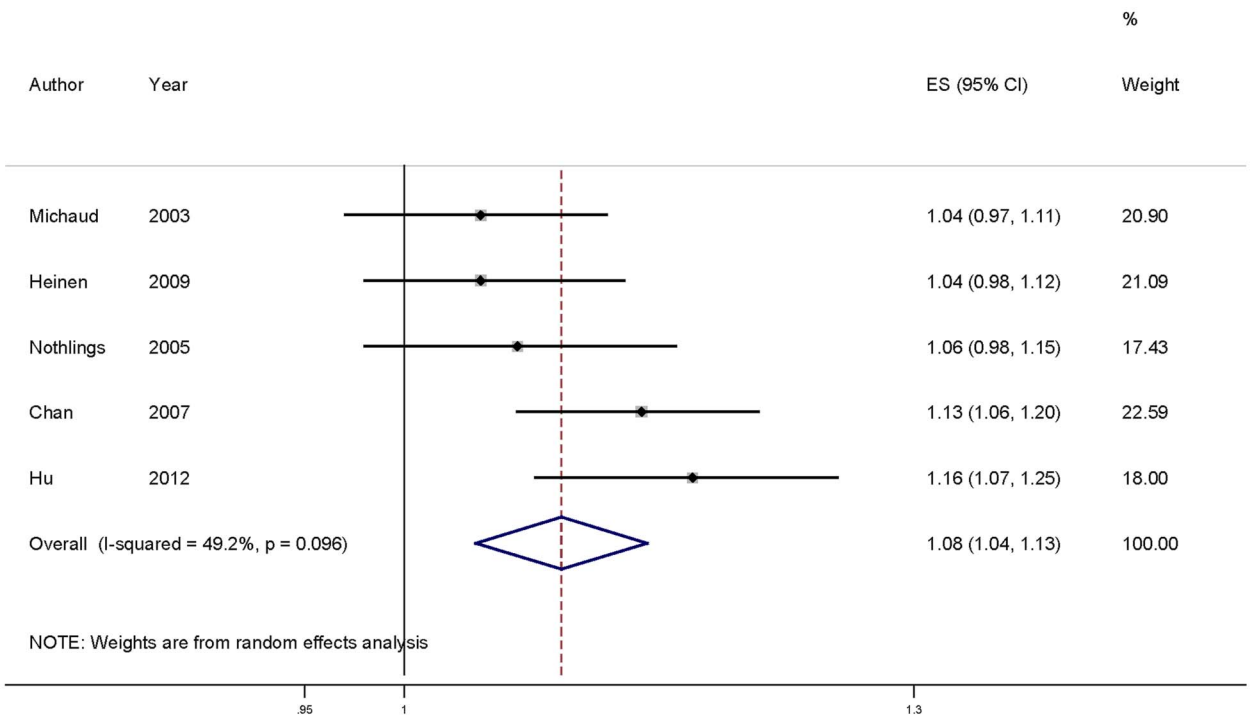

Figure 3 | Dose-response meta-analyses of every 100 mg/day increased intake of cholesterol and the risk of pancreatic cancer. Squares represent study-specific RR, horizontal lines represent 95%CI and diamonds represent summary relative risks.

1. Siegel, R., Ma, J., Zou, Z. & Jemal, A. Cancer statistics, 2014. *CA Cancer J Clin* **64**, 9–29 (2014).
2. Ahrendt, S. A. & Pitt, H. A. Surgical management of pancreatic cancer. *Oncology (Williston Park)* **16**, 725–34; discussion 34, 36–8, 40, 43 (2002).
3. Wu, C. *et al.* Genome-wide association study of survival in patients with pancreatic adenocarcinoma. *Gut* **63**, 152–60 (2014).
4. Tang, H. *et al.* Genes-environment interactions in obesity- and diabetes-associated pancreatic cancer: a GWAS data analysis. *Cancer Epidemiol Biomarkers Prev* **23**, 98–106 (2014).
5. Maisonneuve, P. & Lowenfels, A. B. Epidemiology of pancreatic cancer: an update. *Dig Dis* **28**, 645–56 (2010).
6. Aune, D. *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* **23**, 843–52 (2012).
7. Lucenteforte, E. *et al.* Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann Oncol* **23**, 374–82 (2012).
8. Ben, Q. *et al.* Diabetes mellitus and risk of pancreatic cancer: A meta-analysis of cohort studies. *Eur J Cancer* **47**, 1928–37 (2011).
9. Magruder, J. T., Elahi, D. & Andersen, D. K. Diabetes and pancreatic cancer: chicken or egg? *Pancreas* **40**, 339–51 (2011).
10. Johansen, D. *et al.* Metabolic factors and the risk of pancreatic cancer: a prospective analysis of almost 580,000 men and women in the Metabolic Syndrome and Cancer Project. *Cancer Epidemiol Biomarkers Prev* **19**, 2307–17 (2010).
11. Greenland, S. & Longnecker, M. P. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* **135**, 1301–9 (1992).
12. Orsini, N. & Bellocco, R. Generalized least squares for trend estimation of summarized dose-response data. *Stata J* **6**, 40–57 (2006).
13. Orsini, N. *et al.* Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. *Am J Epidemiol* **175**, 66–73 (2012).
14. Larsson, S. C., Giovannucci, E. & Wolk, A. Folate and risk of breast cancer: a meta-analysis. *J Natl Cancer Inst* **99**, 64–76 (2007).
15. Wang, Z. M. *et al.* Black and green tea consumption and the risk of coronary artery disease: a meta-analysis. *Am J Clin Nutr* **93**, 506–15 (2011).
16. Higgins, J. P. & Thompson, S. G. Quantifying heterogeneity in a meta-analysis. *Stat Med* **21**, 1539–58 (2002).
17. Higgins, J. P. & Thompson, S. G. Controlling the risk of spurious findings from meta-regression. *Stat Med* **23**, 1663–82 (2004).
18. Tobias, A. Assessing the in fluence of a single study in the meta-analysis estimate. *Stata Tech Bull* **47**, 15–7 (1999).
19. Egger, M., Davey Smith, G., Schneider, M. & Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **315**, 629–34 (1997).
20. Howe, G. R., Jain, M. & Miller, A. B. Dietary factors and risk of pancreatic cancer: results of a Canadian population-based case-control study. *Int J Cancer* **45**, 604–8 (1990).
21. Baghurst, P. A. *et al.* A case-control study of diet and cancer of the pancreas. *Am J Epidemiol* **134**, 167–79 (1991).
22. Bueno de Mesquita, H. B., Maisonneuve, P., Runia, S. & Moerman, C. J. Intake of foods and nutrients and cancer of the exocrine pancreas: a population-based case-control study in The Netherlands. *Int J Cancer* **48**, 540–9 (1991).
23. Zatonski, W. *et al.* Nutritional factors and pancreatic cancer: a case-control study from south-west Poland. *Int J Cancer* **48**, 390–4 (1991).
24. Olsen, G. W. *et al.* Nutrients and pancreatic cancer: a population-based case-control study. *Cancer Causes Control* **2**, 291–7 (1991).
25. Howe, G. R. *et al.* A collaborative case-control study of nutrient intake and pancreatic cancer within the search programme. *Int J Cancer* **51**, 365–72 (1992).
26. Kalapothaki, V. *et al.* Nutrient intake and cancer of the pancreas: a case-control study in Athens, Greece. *Cancer Causes Control* **4**, 383–9 (1993).
27. Ghadirian, P., Baillargeon, J., Simard, A. & Perret, C. Food habits and pancreatic cancer: a case-control study of the Francophone community in Montreal, Canada. *Cancer Epidemiol Biomarkers Prev* **4**, 895–9 (1995).
28. Stolzenberg-Solomon, R. Z. *et al.* Prospective study of diet and pancreatic cancer in male smokers. *Am J Epidemiol* **155**, 783–92 (2002).
29. Michaud, D. S. *et al.* Dietary meat, dairy products, fat, and cholesterol and pancreatic cancer risk in a prospective study. *Am J Epidemiol* **157**, 1115–25 (2003).
30. Nothlings, U. *et al.* Meat and fat intake as risk factors for pancreatic cancer: the multiethnic cohort study. *J Natl Cancer Inst* **97**, 1458–65 (2005).
31. Lin, Y. *et al.* Nutritional factors and risk of pancreatic cancer: a population-based case-control study based on direct interview in Japan. *J Gastroenterol* **40**, 297–301 (2005).
32. Chan, J. M., Wang, F. & Holly, E. A. Pancreatic cancer, animal protein and dietary fat in a population-based study, San Francisco Bay Area, California. *Cancer Causes Control* **18**, 1153–67 (2007).
33. Heinen, M. M., Verhage, B. A., Goldbohm, R. A. & van den Brandt, P. A. Meat and fat intake and pancreatic cancer risk in the Netherlands Cohort Study. *Int J Cancer* **125**, 1118–26 (2009).
34. Lucenteforte, E. *et al.* Macronutrients, fatty acids, cholesterol and pancreatic cancer. *Eur J Cancer* **46**, 581–7 (2010).
35. Hu, J. *et al.* Dietary cholesterol intake and cancer. *Ann Oncol* **23**, 491–500 (2012).
36. Ferretti, G. *et al.* Structural modifications of HDL and functional consequences. *Atherosclerosis* **184**, 1–7 (2006).
37. Haddy, N. *et al.* IL-6, TNF-alpha and atherosclerosis risk indicators in a healthy family population: the STANISLAS cohort. *Atherosclerosis* **170**, 277–83 (2003).
38. Mainous, A. G., 3rd *et al.* Iron, lipids, and risk of cancer in the Framingham Offspring cohort. *Am J Epidemiol* **161**, 1115–22 (2005).
39. Wells, B. J., Mainous, A. G., 3rd, Everett, C. J. & Gill, J. M. Iron, cholesterol, and the risk of cancer in an 18-year cohort. *Asian Pac J Cancer Prev* **6**, 505–9 (2005).

40. Prentice, R. L. Dietary assessment and the reliability of nutritional epidemiology reports. *Lancet* **362**, 182–3 (2003).
41. Willett, W. C. *et al.* Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* **122**, 51–65 (1985).

Author contributions

Hongqiang Chen, Shiyong Qin, Minghai Wang, Tao Zhang, Shuguang Zhang, HQC and SYQ designed the experiments; SYC, MHW and TZ collected the data; HQC and SGZ wrote the main manuscript text and all authors reviewed the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Chen, H., Qin, S., Wang, M., Zhang, T. & Zhang, S. Association between cholesterol intake and pancreatic cancer risk: Evidence from a meta-analysis. *Sci. Rep.* **5**, 8243; DOI:10.1038/srep08243 (2015).