科技部補助專題研究計畫成果報告
期末報告

慢性感染與糖尿病和代謝症候群之世代研究

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 104-2314-B-041-002-
執 行 期 間 ： 104年08月01日至105年07月31日
執 行 單 位 ： 嘉藥學校財團法人嘉南藥理大學職業安全衛生系(含產業安全衛生與防災碩士班)

計 畫 主 持 人： 魏榮男
共 同 主 持 人： 李弘元
計畫參與人員： 學士級-專任助理人員：許心華
學士級-專任助理人員：廖瑩竹
大專生-兼任助理人員：蕭琦真

中 華 民 國 105 年 09 月 13 日

中 文 摘 要 ： 目的：探討胃萎縮、幽門螺旋桿菌感染、B、C 型肝炎與糖尿病發生的關係
研究設計: 本世代研究以台灣台大雲林分院建立的世代(Taiwan Lifestyle Study)做為研究對象, 包含從西元2006至2012的所有樣本,平均追蹤了3.4±1.6 年。
參與樣本:共有1397人在第一年檢查診斷時沒有糖尿病做為追蹤的對象，追蹤成功的有855人(62%)。
測量方法與結果: 糖尿病的診斷標準是依據葡萄糖耐性測試與 hemoglobin A1c 和是否服用糖尿病藥物。幽門螺旋桿菌感染是以血清中的分析幽門螺旋桿菌的IgG抗體，並使用pepsinogen 1(PG-1)、pepsinogen 2(PG-2)及兩者的比例(PG-1/PG-2)來代表胃黏膜萎縮的狀態，胃萎縮的定義為血清中 pepsinogen (PG) I 70 ng/mL 與 PG I/II ratio 3。
結果: 追蹤成功的樣本中，有283人 (36%) 幽門螺旋桿菌感染陽性、78(9%)人被診斷為胃萎縮。血清中PG I/II比例與 HOMA2-IR、HOMA2%B和H. pylori IgG titer 呈現負相關(all p<0.05)，世代持續追蹤後，共73人(9%)罹患糖尿病。發現胃萎縮的病人的糖尿病發生率比較低(HR 0.28, 95% CI 0.09-0.91, p<0.05)，校正的干擾因自有年齡、性別、體質比、 log-transformed HOMA2-IR, log-transformed HOMA2%B, 糖尿病家族史、糖化血色素、血壓、三酸甘油脂與總膽固醇。血清中的PG I/II比例也可以預測糖尿病的發生(adjusted HR 2.06, 95% CI 1.11-3.84, p<0.05)；然而，幽門螺旋桿菌的感染就與糖尿病的發生沒有統計相關 (p>0.05)。B、C 型肝炎感染與糖尿病發生也沒有統計相關。
結論： 胃萎縮與糖尿病的發生有關，但幽門螺旋桿菌感染則無統計相關，B、C 型肝炎也沒有。此發現認為胃萎縮對於糖尿病發生反而是個保護因子。

中 文 關 鍵 詞 ： 糖尿病、B型肝炎、C型肝炎、幽門螺旋桿菌、胃萎縮、世代

英 文 摘 要 ： Objective: To examine the relationship among gastric atrophy, H. pylori infection,
hepatitis B/C infection and incident diabetes.
Design: A cohort study, named as the Taiwan Lifestyle Study, which included participants from 2006 to 2012, and followed them for an average of 3.4±1.6 years for incident diabetes.
Participants: We included 1379 subjects without diabetes at baseline. Among them, 855 subjects (62%) were successfully followed.
Main Outcomes and Measures: Diabetes was diagnosed by the results of oral glucose tolerance tests and hemoglobin A1c, and the use of medications for diabetes. Serum IgG antibodies against H. pylori were measured at baseline. Gastric atrophy was defined as serum pepsinogen (PG) I ?70 ng/mL and PG I/II ratio ?3. PG I/II ratio was used as a measure for the extent of gastric atrophy.
Results: At baseline, 283 (36%) subjects were positive for

H. pylori infection and 78 (9%) subjects were diagnosed as gastric atrophy. Serum PG I/II ratio was inversely correlated with HOMA2-IR, HOMA2%B, and H. pylori IgG titer (all p<0.05). There were 130 subjects with hepatitis B and 38 with hepatitis C. During follow-up, 73 subjects (9%) developed diabetes. Subjects with gastric atrophy had a lower risk of incident diabetes (HR 0.28, 95% CI 0.09-0.91, p<0.05), adjusting for age, gender, body mass index, log-transformed HOMA2-IR, log-transformed HOMA2%B, family history of diabetes, Hemoglobin A1c, systolic blood pressure, diastolic blood pressure, log-transformed triglyceride, and total cholesterol. Serum PG I/II ratio predicted incident diabetes (adjusted HR 2.06, 95% CI 1.11-3.84, p<0.05). However, H. pylori infection was not associated with incident diabetes (p>0.05). There were not significant relationship between hepatitis B/C and diabetes.
Conclusions and Relevance: The presence and the extent of gastric atrophy, but not H. pylori infection and hepatitis B/C infection, are associated with incident diabetes. The findings suggest that gastric atrophy is a protective factor for the incidence of diabetes.

英文關鍵詞：diabetes, hepatitis B, hepatitis C, helicobacter pylori, gastric atrophy, cohort

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

慢性感染與糖尿病和代謝症候群之世代研究

計畫類別：■個別型計畫　□整合型計畫
計畫編號：MOST 104-2314-B-041-002 –
執行期間： 104 年 08 月 01 日至 105 年 07 月 31 日

執行機構及系所：嘉南藥理大學職業安全衛生系

計畫主持人：魏榮男
共同主持人：李弘元
計畫參與人員：廖瑩竹、許心華、蕭琦真

本計畫除繳交成果報告外，另含下列出國報告，共 ___ 份：
□執行國際合作與移地研究心得報告
□出席國際學術會議心得報告

期末報告處理方式：

1. 公開方式：
   □非列管計畫亦不具下列情形，立即公開查詢
   ■涉及專利或其他智慧財產權，□一年■二年後可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：■否 □是
3.「本報告」是否建議提供政府單位施政參考 ■否 □是，____（請列舉提供之單位；本部不經審議，依勾選逕予轉送）

中　華　民　國 105　年　08　月　30　日

Introduction

Diabetes mellitus and its complications are global threats in public health nowadays. Since there is still much to be improved in the treatment of diabetes and its complications, prevention or delay of the development of diabetes is an important strategy to fight against the diabetes epidemic. To achieve this, identification of treatable causes is a key and the first step for success.

Gastric atrophy is characterized by chronic inflammation of gastric mucosa with loss of gastric glandular cells. In practice, serum pepsinogen (PG) I and II can be used as a screening tool for gastric atrophy, defined by a low serum PG I (≤70 ng/mL) and a low PG I/II ratio (≤3). A decreased serum PG I level or PG I/II ratio not only reflects an increasing extent of gastric atrophy,[1,2] but also correlates with a decreased plasma ghrelin [3], which is an important gut hormone involved in glucose homeostasis.[4] Besides, gastric acid secretion is decreased in subjects with gastric atrophy. Decreased gastric acid secretion can change the compositions of gut microbiota [5,6] and influence dietary nutrient absorption.[7] In addition, gastric atrophy may interfere with the absorption of vitamin B12, which also plays a role in carbohydrate and fat metabolism.[8] Vitamin B12 deficiency in pregnancy is associated with the incidence of gestational diabetes and diabetes after delivery.[9] Taken together, these reports suggest that gastric atrophy may be associated with the incidence of diabetes.

Among various causes of gastric atrophy, *Helicobactor pylori* (*H. pylori*) infection is a major one. It is estimated that approximately 50% of world's population have been infected with *H. pylori*.[10] *H. pylori* infection results in various gastrointestinal disorders, such as chronic gastritis and peptic ulcer.[11-13] Besides, *H. pylori* infection also correlates to metabolic syndrome, an important risk factor of diabetes.[14] Some epidemiological studies indicate a higher prevalence of *H. pylori* infection in patients with diabetes.[15-17] In cross-sectional studies, *H. pylori* infection has been associated with insulin secretion, HbA1c, and diabetes.[18,19] There is only one prospective study which investigated the influence of *H. pylori* infection on the incidence of diabetes. In the report, *H. pylori* infection was associated with an increased incidence of diabetes in a Latino elderly cohort.[20] However, controlling for markers of systemic inflammation including C-reactive protein and interleukin-6 did not attenuate the effect of *H. pylori* infection, suggesting that factors other than systemic inflammation, such as above-mentioned changes in gastric atrophy, may contribute to the link between *H. pylori* infection and the incidence of diabetes.

To the best of our knowledge, there is no study which investigates the relationship of gastric atrophy to the incidence of diabetes. Besides, previous studies examining the association between *H. pylori* infection and diabetes did not consider the effect of gastric atrophy, and most available data come from cross-sectional

studies. Therefore, we conducted a community-based, prospective cohort study to investigate the relationship between *H. pylori* infection, gastric atrophy, and the incidence of diabetes.

Research design and methods

Patients

We derived the data for analyses from the Taiwan Lifestyle Study, a large prospective cohort study.[21] From 2006 to 2012, individuals aged 18 years and above who had undergone health examinations at the National Taiwan University Hospital Yun-Lin branch in the previous year and had fasting plasma glucose <126 mg/dL (7.0 mmol/L) were invited in this cohort study. All subjects were evaluated by questionnaires, undergoing physical examinations and blood tests including oral glucose tolerance tests, with the aid of trained nurses. All study subjects were contacted by telephone, e-mail, or postal mail 1-3 years after the initial visit and every 2 years thereafter. Follow-up visits were scheduled according to the respondent's availability.

All subjects diagnosed with diabetes during follow-up were classified as type 2 diabetes by endocrine specialists based on their body mass index (BMI), family history of diabetes, age, and the fact that no one had diabetic ketoacidosis. In this

study, we excluded the following patients from the analysis: 1) individuals with previously diagnosed diabetes or who met the diagnostic criteria of diabetes at enrollment, 2) individuals with missing information for serum PG levels and *H. pylori*-specific antibody titers, and 3) individuals who failed to be followed. Written informed consent was obtained from each participant. The study was reviewed and approved by the institutional review board.

Measurements and assays

Blood samples were collected from each participant after overnight fasting and then stored at -70℃ for serological assay. A standard oral 75-g glucose tolerance test (OGTT) was performed to measure fasting and 2-hour plasma glucose. Plasma glucose, lipid profiles, uric acid, and high-sensitive C-reactive protein (hsCRP) were measured with an automatic analyzer (Toshiba TBA 120FR, Toshiba Medical Systems Co., Ltd., Tokyo, Japan). Hemoglobin A1c (HbA1c) was measured by an automatic analyzer (HLC-723 G7 HPLC system, Tosoh Corporation, Tokyo, Japan). The HbA1c assay was certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications Trial (DCCT) reference assay. Plasma insulin was measured by an automatic analyzer using microparticle enzyme immunoassay (Abbott AxSYM system; Abbott Laboratories, Abbott Park, IL). Updated computer models for homeostasis model assessment were

used to calculate the indices of insulin resistance (HOMA2-IR) and insulin secretion (HOMA2%B). *H. pylori*-specific immunoglobulin G (Ig G) antibody titers were measured using a commercially enzyme-linked immunosorbent assay assay (R-Biopharm AG, Darmstadt, Germany). Serum PG levels were measured by chemiluminescent enzyme immunoassay (Eiken Chemical Tokyo, Japan).

Definitions

Diabetes was defined as fasting plasma glucose ≥126 mg/dL (7.0 mmol/L), OGTT 2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L), HbA1c ≥6.5% (48 mmol/mol), or receiving medications for diabetes.[22] *H. pylori*-specific IgG antibody titers ≥16 U/mL, 10-16 U/mL, and <10 U/mL was defined as positive, borderline positive, and negative for *H. pylori* infection, respectively. Gastric atrophy was defined as PG I ≤70 ng/mL and PG I/II ratio ≤3. PG I/II ratio was used as a measure of the extent of gastric atrophy.

Statistical analyses

Sample size estimation for *H. pylori* infection to predict the incidence of diabetes was done with the following data. The prevalence of *H. pylori* infection has been reported to be 50-80%.[23] The incidence of diabetes in the present cohort has been reported to be 2.92%.[21] In a follow-up period of 3.4 years, there will be 9.9212% of study subjects with incident diabetes. The hazard ratio (HR) for incident diabetes in

subjects with *H. pylori* infection has been reported to be 2.69.[20] Therefore, the sample size needed to detect a difference in the incidence of diabetes between subjects with and without *H. pylori* infection at a two-sided alpha level of 0.05 and power of 80% is 324 when the prevalence of *H. pylori* infection is 50% or 505 when the prevalence of *H. pylori* infection is 80%. Continuous variables with normal distribution were presented as means ± standard deviation (SD). Variables with skewed distribution were presented as medians (interquartile ranges) and were analyzed after logarithmic transformation. The statistical significance of the differences in different subgroups was tested with Student's *t* test, Fisher's exact test or χ^2 test. Unadjusted Pearson's correlation coefficients and partial correlation coefficients, adjusting for age and gender, were used to test the relationship between serum PG I/II ratio and other variables. We performed Cox proportional hazards models to estimate the hazard ratios of determinants for the incidence of diabetes. Variables significantly associated with the incidence of diabetes in univariate Cox proportional hazards models and clinically important variables were included in multivariate analyses. The incidence of diabetes in subgroups was estimated by the Kaplan-Meier method and was tested by log-rank tests. A two-tailed *p*-value below 0.05 was considered significant. Stata/SE 14.0 for Windows (StataCorp LP, College Station, TX) was used for statistical analyses.

Results

At baseline, there were 1379 individuals who were not diagnosed as diabetes. Among them, 855 subjects (62%) were successfully followed. There was no significant difference between participants who were successfully followed and who were lost to follow-up (Supplementary table 1). Among the 855 subjects who were successfully followed, 306 (36%) subjects were positive for *H. pylori* infection and 78 (9%) subjects were diagnosed as gastric atrophy at baseline. During a 3.4±1.6 years of follow-up, 73 subjects developed type 2 diabetes. Table 1 presents the baseline characteristics of these subjects according to diabetes status at the end of follow-up. Compared with subjects who did not develop diabetes, those with incident diabetes were older and had higher levels of BMI, systolic blood pressure, fasting plasma glucose, OGTT 2-h plasma glucose, HbA1c, HOMA2-IR, total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, and hsCRP.

Table 2 shows the correlations between serum PG I/II ratio with *H. pylori*-specific IgG antibody titers and other important risk factors of diabetes. In bivariate Pearson's correlation tests, serum PG I/II ratio was inversely correlated with age, BMI, HOMA2-IR, and *H. pylori* IgG titer. The negative relationship of serum PG I/II ratio to HOMA2-IR and *H. pylori* IgG titer remained significant after adjusting for

age and gender ($p<0.05$). Neither fasting glucose, OGTT 2-h glucose, HbA1c, nor hsCRP showed any significant association with serum PG I/II ratio (all $p>0.05$). Although the bivariate correlation coefficient between serum PG I/II ratio and HOMA2%B did not reach statistical significance, the partial correlation test confirmed this negative relationship after adjusting for age and gender (partial correlation coefficient r=-0.0778, p=0.0233). Serum PG I/II ratio was similar in subjects with and without family history of diabetes (4.8 vs. 4.6, P=0.2681).

We used Cox proportional hazards models to calculate the HRs of *H. pylori* infection, serum PG I/II ratio, and gastric atrophy to predict the incidence of diabetes (Table 3). In univariate analyses, serum PG I/II ratio was associated with the incidence of diabetes with borderline significance (HR 1.84 [95% CI 0.94-3.60], p=0.073). Subjects with serum PG I/II ratio in the middle and the highest (borderline significance) tertile had a higher risk of incident diabetes compared with those in the lowest tertile (the middle tertile, HR 1.87 [95% CI 1.02-3.42], $p<0.05$; the highest tertile, HR 1.86 [95% CI 0.99-3.47], p=0.052). In model 1, serum PG I/II ratio was significantly associated with incident diabetes, adjusted for age, gender, and BMI (HR 2.14 [95% CI 1.12-4.11], $p<0.05$). The HRs for incident diabetes in the middle and highest tertile of serum PG I/II ratio were 2.13 (95% CI 1.16-3.90, $p<0.05$) and 2.15 (95% CI 1.15-4.04, $p<0.05$), respectively in this model. The association between

serum PG I/II ratio and incident diabetes remained significant after further adjustment for family history of diabetes, fasting plasma glucose, log-transformed HOMA2-IR, log-transformed HOMA2%B (model 2), family history of diabetes, HbA1c, blood pressure, and lipid profiles (model 3). For gastric atrophy, it was borderline significantly associated with incident diabetes in model 1 and was significantly associated with incident diabetes in model 2 and 3 (model 1, HR 0.35 [95% CI 0.11-1.12], p=0.076; model 2 HR 0.28 [95% CI 0.08-0.90], p<0.05; model 3, HR 0.28 [95% CI 0.09-0.91], p<0.05). Serum PG I/II ratio and gastric atrophy remained significantly associated with incident diabetes when *H. pylori* infection was introduced as a covariate (serum PG I/II ratio, adjusted HR 2.21 [95% CI 1.10-4.46], p<0.05; gastric atrophy, adjusted HR 0.29 [95% CI 0.09-0.97], p<0.05; both adjusted for age, gender, body mass index, log-transformed HOMA2-IR, log-transformed HOMA2%B, family history of diabetes, HbA1c, systolic blood pressure, diastolic blood pressure, log-transformed triglyceride, total cholesterol, and *H. pylori* infection). On the other hand, neither *H. pylori* serostatus nor serum PG I concentrations were associated with the incidence of diabetes in univariate and multivariate models. The findings remained constant after further adjustment for gastric atrophy. Moreover, in our study, 665 subjects had follow-up data on *H. pylori* serostatus at the last visit. Among these subjects, there were 26 subjects with *H. pylori* sero-conversion, which

was defined as a change from a positive or borderline positive to a negative serologic test result. After excluding these 26 subjects with sero-conversion, there was no significant association between *H. pylori* infection and the incidence of diabetes, either (HR 1.00, 95% CI 0.99-1.00, p=0.608).

In Figure 1, we divided the population into two categories according to tertile of serum PG I/II ratio as follows: group 1 = the lowest tertile, serum PG I/II ratio <4.125; group 2 = the combination of the middle and the highest tertile, serum PG I/II ratio≥4.125. The Kaplan-Meier curve demonstrated that subjects with a lower serum PG I/II ratio, which means more extensive gastric atrophy, had a lower risk of incident diabetes ($p<0.05$ by log-rank test).

Discussion

In this prospective cohort study, we demonstrated that subjects with gastric atrophy had a lower risk of incident diabetes, compared to those without gastric atrophy. The extent of gastric atrophy, measured by serum PG I/II ratio, was correlated with age, *H. pylori* IgG titer, HOMA2-IR, and HOMA2%B. When gastric atrophy is more extensive, presented as a lower serum PG I/II ratio, the risk of incident diabetes is lower. On the other hand, there was no significant association between *H. pylori* infection and the incidence of diabetes.

To our best knowledge, this is the first paper that shows gastric atrophy predicts the incidence of diabetes. In a cross-sectional study, Tanaka *et al*[24] reported that serum PG I/II ratio was positively related to several metabolic parameters, including plasma glucose, triglyceride, and uric acid levels. By contrast, we did not find a significant association between serum PG I/II ratio and plasma glucose concentration at baseline in the present study. Instead, serum PG I/II ratio was negatively correlated with both HOMA2-IR and HOMA2%B. Since the improved insulin sensitivity with increasing serum PG I/II ratio may be offset by the attenuated β-cell function, the negative association of serum PG I/II ratio to HOMA2-IR and HOMA2%B can explain why serum PG I/II ratio was not associated with plasma glucose concentration.

The mechanism by which gastric atrophy affects the risk of diabetes is unclear. The lack of ghrelin secretion in gastric atrophy could be one possibility. Ghrelin is a pleiotropic hormone that is involved in appetite control, energy metabolism, and glucose homeostasis. Ghrelin can stimulate food intake,[25] increase fat mass,[26] stimulate growth hormone secretion,[27] reduce glucose-stimulated insulin secretion,[28,29] and induce hyperglycemia. It has been reported that plasma ghrelin levels correlated positively with both serum PG I and serum PG I/II ratio.[3] In other words, plasma ghrelin levels decreased with increasing extent of gastric atrophy. Therefore, low plasma ghrelin levels in subjects with gastric atrophy may explain why

gastric atrophy predicts a lower incidence of diabetes in this study. Besides, gastric acid secretion is decreased in subjects with gastric atrophy. Decreased gastric acid secretion can change the compositions of gut microbiota[5,6] and influence dietary nutrient absorption.[7] Therefore, it is reasonable to hypothesize that gastric atrophy may decrease diabetes risk through the interaction of host and gut microbiota, which is worthwhile to explore in future researches.

The relationship between *H. pylori* infection and diabetes remains controversial. Several studies support our findings on the lack of association between *H. pylori* infection and diabetes. It has been shown that the rate of *H. pylori* infection was similar between subjects with and without diabetes in Hong Kong Chinese population.[30] The prevalence of diabetes is not related to *H. pylori* sero-positivity after adjustment for demographic factors.[31] In contrast, Hsieh *et al.*[18] found that *H. pylori* infection was significantly associated with higher HbA1c and decreased insulin secretion in Taiwanese population. However, these reports are all cross-sectional design, the temporal relationship between *H. pylori* infection and diabetes cannot be clarified. By contrast, the present study is a prospective longitudinal cohort study. *H. pylori* infection was detected before the incidence of diabetes. With this design, there is no significant association between *H. pylori* serostatus and incident diabetes. However, another longitudinal study by Jeon *et al.*[20] has demonstrated that *H. pylori*

infection leads to an increased incidence of diabetes in Latino elderly population. The discrepant findings may result from race or ethnic difference, different prevalence of *H. pylori* infection and gastric atrophy, and different age groups studied. In the Latino elderly study, 93% of the study subjects were sero-positive for *H. pylori* infection, whereas in the present study, only 36% of the study subjects were sero-positive for *H. pylori* infection. The average age of the study subjects were 68.7 years in the Latino elderly study, while the average age was 49.3 years in the present study. Besides, since there was no report on the prevalence of gastric atrophy in Latino study, we cannot compare the differences between the two studies. Taken together, further longitudinal studies from different populations are needed, in order to understand the interaction of race or ethnicity, prevalence of *H. pylori* infection and gastric atrophy, and age on the relationship between *H. pylori* infection and the incidence of diabetes.

This is the first prospective study to investigate the role of gastric atrophy on the incidence of diabetes. The longitudinal study design allows us to accurately clarify the temporal relationships between gastric atrophy, *H. pylori* infection, and the incidence of diabetes. By contrast, our study has some limitations. First, we do not have the information on *H. pylori* eradication, which may affect glucose metabolism and insulin sensitivity[32,33]. However, some patients in our study had follow-up data on *H. pylori* serostatus at the last visit. After excluding those with sero-conversion,

non-significant association between *H. pylori* infection and the incidence of diabetes remained constant (see Results for detail numbers). Therefore, it is reasonable to speculate that *H. pylori* eradication has limited influence on our findings. Second, we did not measure plasma ghrelin levels which have been reported to be related to both gastric atrophy and the incidence of diabetes. Third, generalization of the findings to other populations may be limited because all the subjects in the present study were Han Chinese.

Conclusions

We have demonstrated that subjects with gastric atrophy are associated with a lower incidence of diabetes. When gastric atrophy is more extensive, presented as a lower serum PG I/II ratio, the risk of incident diabetes is lower. By contrast, there is no significant association between *H. pylori* infection and the incidence of diabetes. Further studies are needed to investigate the detailed mechanisms and the potential applications of the findings to guide diabetes screening and treatment strategies.

Acknowledgements

Author contributions: Dr Li had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Liou, Wu, Li.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Yu, Li.

Statistical analysis: Yu, Wei, Li.

Obtained funding: Wu, Li.

Administrative, technical, or material support: All authors.

Study supervision: Wu, Li.

Conflict of Interest Disclosures: All authors do not have any conflicts of interest.

Funding/Support: This work is funded by a grant (DAROC2014YPI-0001) from the Diabetes Association of Republic of China, Taiwan.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and decision to submit the manuscript for publication.

Additional Contributions: The authors would like to thank Miss Chien-Yin Su, Miss Ying-Jhu Liao, Miss Kuan-Yi Wu, and the staff of the eighth Core Lab, Department of Medical Research, National Taiwan University Hospital for their technical and

computing assistance.

References

1. Miki K, Ichinose M, Shimizu A, et al. Serum pepsinogens as a screening test of extensive chronic gastritis. *Gastroenterol Jpn.* 1987;22(2):133-141.

2. Miki K, Ichinose M, Ishikawa KB, et al. Clinical application of serum pepsinogen I and II levels for mass screening to detect gastric cancer. *Jpn J Cancer Res.* 1993;84(10):1086-1090.

3. Suzuki H, Masaoka T, Hosoda H, et al. Plasma ghrelin concentration correlates with the levels of serum pepsinogen I and pepsinogen I/II ratio--a possible novel and non-invasive marker for gastric atrophy. *Hepatogastroenterology.* 2004;51(59):1249-1254.

4. Delhanty PJ, van der Lely AJ. Ghrelin and glucose homeostasis. *Peptides.* 2011;32(11):2309-2318.

5. Li XX, Wong GL, To KF, et al. Bacterial microbiota profiling in gastritis without Helicobacter pylori infection or non-steroidal anti-inflammatory drug use. *PLoS One.* 2009;4(11):e7985.

6. Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci U S A.* 2006;103(3):732-737.

7. Krasinski SD, Russell RM, Samloff IM, et al. Fundic atrophic gastritis in an

elderly population. Effect on hemoglobin and several serum nutritional indicators. *J Am Geriatr Soc.* 1986;34(11):800-806.

8. Chow BF, Stone HH. The relationship of vitamin B12 to carbohydrate metabolism and diabetes mellitus. *Am J Clin Nutr.* 1957;5(4):431-439.

9. Krishnaveni GV, Hill JC, Veena SR, et al. Low plasma vitamin B12 in pregnancy is associated with gestational 'diabesity' and later diabetes. *Diabetologia.* 2009;52(11):2350-2358.

10. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol Rev.* 2000;22(2):283-297.

11. Goodwin CS, Mendall MM, Northfield TC. Helicobacter pylori infection. *Lancet.* 1997;349(9047):265-269.

12. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med.* 1977;62(5):707-714.

13. Weck MN, Gao L, Brenner H. Helicobacter pylori infection and chronic atrophic gastritis: associations according to severity of disease. *Epidemiology.* 2009;20(4):569-574.

14. Gunji T, Matsuhashi N, Sato H, et al. Helicobacter pylori infection is significantly associated with metabolic syndrome in the Japanese population.

Am J Gastroenterol. 2008;103(12):3005-3010.

15. Bener A, Micallef R, Afifi M, Derbala M, Al-Mulla HM, Usmani MA. Association between type 2 diabetes mellitus and Helicobacter pylori infection. *Turk J Gastroenterol.* 2007;18(4):225-229.

16. Gulcelik NE, Kaya E, Demirbas B, et al. Helicobacter pylori prevalence in diabetic patients and its relationship with dyspepsia and autonomic neuropathy. *J Endocrinol Invest.* 2005;28(3):214-217.

17. Devrajani BR, Shah SZ, Soomro AA, Devrajani T. Type 2 diabetes mellitus: A risk factor for Helicobacter pylori infection: A hospital based case-control study. *Int J Diabetes Dev Ctries.* 2010;30(1):22-26.

18. Hsieh MC, Wang SS, Hsieh YT, Kuo FC, Soon MS, Wu DC. Helicobacter pylori infection associated with high HbA1c and type 2 diabetes. *Eur J Clin Invest.* 2013;43(9):949-956.

19. Yang GH, Wu JS, Yang YC, Huang YH, Lu FH, Chang CJ. Gastric Helicobacter pylori infection associated with risk of diabetes mellitus, but not prediabetes. *J Gastroenterol Hepatol.* 2014;29(10):1794-1799.

20. Jeon CY, Haan MN, Cheng C, et al. Helicobacter pylori infection is associated with an increased rate of diabetes. *Diabetes Care.* 2012;35(3):520-525.

21. Ma WY, Yang CY, Shih SR, et al. Measurement of Waist Circumference:

midabdominal or iliac crest? *Diabetes Care.* 2013;36(6):1660-1666.

22. ADA. Standards of Medical Care in Diabetes-2016. *Diabetes Care.* 2016;39:S1-S112.

23. Hunt RH, Xiao SD, Megraud F, et al. Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. *J Gastrointestin Liver Dis.* 2011;20(3):299-304.

24. Tanaka M, Fukui M, Kuroda M, et al. Pepsinogen I/II ratio is related to glucose, triacylglycerol, and uric acid levels. *Nutrition.* 2012;28(4):418-421.

25. Cummings DE. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiol Behav.* 2006;89(1):71-84.

26. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature.* 2000;407(6806):908-913.

27. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature.* 1999;402(6762):656-660.

28. Broglio F, Arvat E, Benso A, et al. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab.* 2001;86(10):5083-5086.

29. Tong J, Prigeon RL, Davis HW, et al. Ghrelin suppresses glucose-stimulated

insulin secretion and deteriorates glucose tolerance in healthy humans. *Diabetes.* 2010;59(9):2145-2151.

30. Ko GT, Chan FK, Chan WB, et al. Helicobacter pylori infection in Chinese subjects with type 2 diabetes. *Endocr Res.* 2001;27(1-2):171-177.

31. Lutsey PL, Pankow JS, Bertoni AG, Szklo M, Folsom AR. Serological evidence of infections and Type 2 diabetes: the MultiEthnic Study of Atherosclerosis. *Diabet Med.* 2009;26(2):149-152.

32. Zojaji H, Ataei E, Sherafat SJ, Ghobakhlou M, Fatemi SR. The effect of the treatment of Helicobacter pylori infection on the glycemic control in type 2 diabetes mellitus. *Gastroenterol Hepatol Bed Bench.* 2013;6(1):36-40.

33. Gen R, Demir M, Ataseven H. Effect of Helicobacter pylori eradication on insulin resistance, serum lipids and low-grade inflammation. *South Med J.* 2010;103(3):190-196.

Table 1. Clinical characteristics of participants at baseline, stratified by follow-up diabetes status.

	Participants who did not develop diabetes	Participants who develop diabetes	*P*
N (%)	782 (91)	73 (9)	
Age (years)	48.7 ± 11.9	54.9 ± 10.2	**<0.0001**
Male (N, %)	275 (35)	31 (42)	0.213
Body mass index (kg/m^2)	23.8 ± 3.4	25.1 ± 2.7	**0.0015**
Family history of diabetes (N, %)	317 (41)	32 (44)	0.577
Systolic blood pressure (mmHg)	122±16	127 ± 17	**0.003**
Diastolic blood pressure (mmHg)	78 ± 10	80 ± 10	0.1588
Fasting plasma glucose (mmol/L)	4.94 ± 0.44	5.33 ± 0.56	**<0.0001**
OGTT 2-h plasma glucose (mmol/L)	6.11 ± 0.11	7.55 ± 1.83	**<0.0001**
HbA1c (mmol/mol)	38 ± 4.4	41 ± 4.4	**<0.0001**
HbA1c (%)	5.6 ± 0.4	5.9 ± 0.4	**<0.0001**
HOMA2-IR	0.75 (0.5-1.1)	1.1 (0.84-1.43)	**<0.0001**
HOMA2%B	80.65 (62.6-104.8)	90.9 (68.6-109.5)	0.2377
Total cholesterol (mmol/L)	5.00 ± 0.91	5.34 ± 0.96	**0.0017**
Triglyceride (mmol/L)	0.99 (0.71-1.44)	1.36 (0.88-1.80)	**0.0002**
HDL cholesterol (mmol/L)	1.35 ± 0.34	1.29 ± 0.31	0.2271
LDL cholesterol (mmol/L)	3.00 ± 0.83	3.34 ± 0.96	**0.0011**
Uric acid (μmol/L)	321 ± 89	345 ± 77	0.0651
hsCRP (nmol/L)	0.76 (0.38-1.43)	1.24 (0.67-2.76)	**0.0005**
H. pylori IgG titer (U/mL)	5 (1.8-38.9)	4.1 (1.6-33.3)	0.5778
H. pylori serostatus			0.699
Negative (N, %)	462 (59)	47 (64)	
Borderline positive (N, %)	37 (5)	3 (4)	
Positive (N, %)	283 (36)	23 (32)	
PG I (ng/mL)	55.7 ± 26.4	59.5 ± 29.4	0.2445
PG II (ng/mL)	13.5 ± 9.3	13.5 ± 10.7	0.9707
PG I/II ratio	4.7 ± 2.0	4.8 ± 1.3	0.5739
Gastric atrophy (N, %)	75 (9)	3 (4)	0.139

Means ± SDs or medians (interquartile ranges) are shown.

Abbreviations: OGTT, oral glucose tolerance test; HbA1c, hemoglobin A1c; HDL,

high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitive C-reactive protein; PG, pepsinogen

Table 2. The relationship between serum pepsinogen I/II ratio and other clinical characteristics.

	r	p	partial *r**	p*
Age (years)	**-0.1444**	**<0.0001**		
Body mass index (kg/m^2)	**-0.0691**	**0.0443**	-0.0663	0.0539
Fasting plasma glucose (mmol/L)	-0.0608	0.0756	-0.0296	0.3877
OGTT 2-h glucose (mmol/L)	-0.0418	0.2220	0.0064	0.8531
HbA1c (%)	-0.0266	0.4380	0.0242	0.4801
HOMA2-IR†	**-0.0873**	**0.0108**	**-0.0905**	**0.0083**
HOMA2%B†	-0.0560	0.1020	**-0.0778**	**0.0233**
hsCRP (nmol/L)†	-0.0269	0.4323	-0.0165	0.6303
H. pylori IgG titer (U/mL)	**-0.4000**	**<0.0001**	**-0.3858**	**<0.0001**

* Adjusted for age and gender

† Log transformed for analysis.

Serum PG I/II ratio was a measure of the extent of gastric atrophy. A lower serum PG I/II ratio stands for a more extensive condition of gastric atrophy. Serum PG I/II ratio was log transformed for analysis. Abbreviations: OGTT, oral glucose tolerance test; HbA1c, hemoglobin A1c; hsCRP, high-sensitive C-reactive protein

Table 3. Hazard ratios (95%CIs) of *H. pylori* infection or gastric atrophy to predict incident diabetes.

	Crude	Model 1	Model 2	Model 3
H. pylori infection				
IgG titer§	0.98 (0.86-1.11)	0.93 (0.82-1.06)	0.90 (0.79-1.02)	0.90 (0.79-1.03)
Serostatus				
Negative	1	1	1	1
Borderline	0.89 (0.28-2.86)	0.79 (0.24-2.55)	0.93 (0.29-3.01)	0.61 (0.18-2.02)
Positive	0.82 (0.50-1.36)	0.70 (0.43-1.17)	0.66 (0.40-1.09)	0.62 (0.37-1.04)
Gastric atrophy				
No	1	**1**	**1**	**1**
Yes	0.40 (0.13-1.27)	0.35 (0.11-1.12)‡	**0.28 (0.08-0.90)†**	**0.28 (0.09-0.91)†**
Serum PG I/II ratio				
Continuous variable§	1.84 (0.94-3.60)‡	**2.14 (1.12-4.11)†**	**2.35 (1.27-4.35)***	**2.06 (1.11-3.84)†**
Tertile category				
Lowest	1	1	1	1
Middle	**1.87 (1.02-3.42)†**	**2.13 (1.16-3.90)†**	**2.34 (1.27-4.32)***	**2.36 (1.26-4.44)***
Highest	1.86 (0.99-3.47)‡	**2.15 (1.15-4.04)†**	**2.32 (1.23-4.38)***	**2.36 (1.24-4.49)***

* $p<0.01$, † $p<0.05$, ‡ $0.05<p<0.10$

§ Log-transformed

Serum PG I/II ratio was used as a measure of the extent of gastric atrophy. A lower serum PG I/II ratio stands for a more extensive condition of gastric atrophy. Abbreviation: PG, pepsinogen

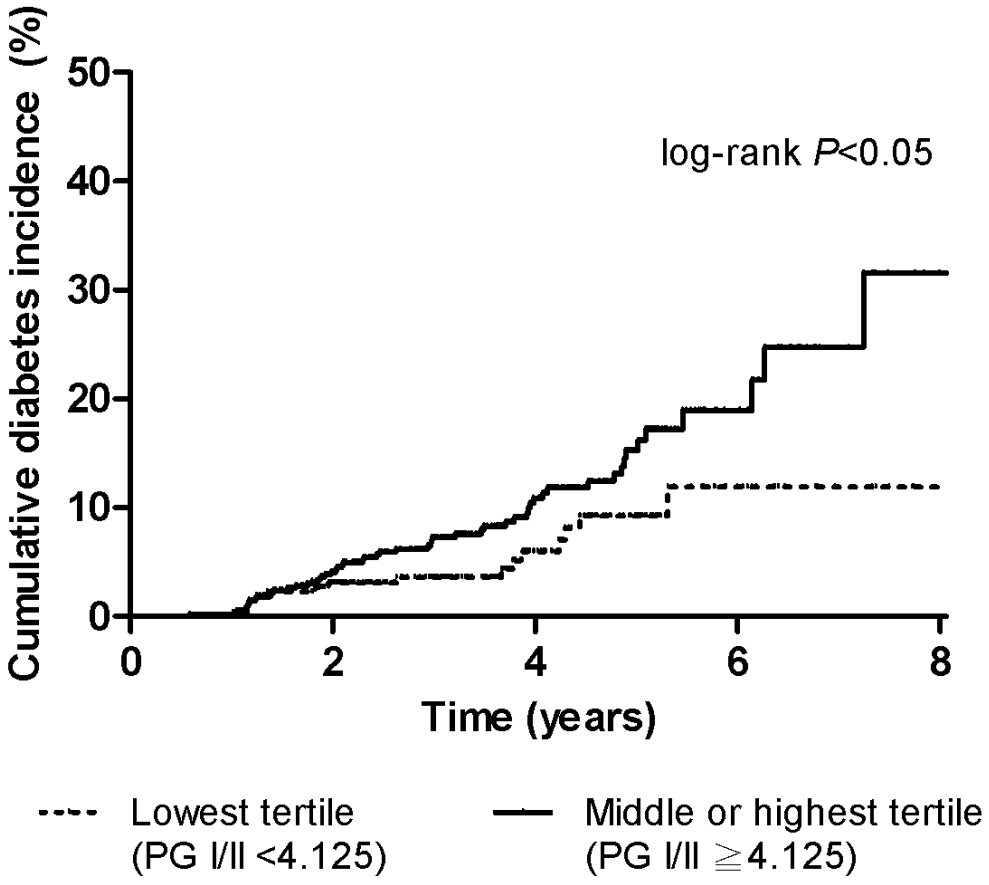
Model 1: adjusted for age, gender, body mass index

Model 2: adjusted for age, gender, body mass index, log-transformed HOMA2-IR, log-transformed HOMA2%B

Model 3: adjusted for age, gender, body mass index, log-transformed HOMA2-IR, log-transformed HOMA2%B, family history of diabetes, HbA1c, systolic blood pressure, diastolic blood pressure, log-transformed triglyceride, and total cholesterol

Figure legends

Figure 1. Kaplan-Meier curve of cumulative diabetes incidence by serum pepsinogen (PG) I/II ratio. Solid line, subjects in the middle or the highest tertile; dashed line, subjects in the lowest tertile.



Supplementary Table 1. Baseline characteristics of participants who were successfully followed and who were lost to follow-up.

	Participants lost to follow-up	Participants who were followed	*P*
N (%)	524 (38%)	855 (62%)	
Age (years)	48.2 ± 13.8	49.3 ± 11.9	0.1469
Male (N, %)	213 (41)	306 (36)	0.071
Body mass index (kg/m^2)	24.1 ± 3.8	23.9 ± 3.3	0.3135
Family history of diabetes (N, %)	186 (35)	349 (40)	0.419
Systolic blood pressure (mmHg)	123 ± 18	122 ± 16	0.3999
Diastolic blood pressure (mmHg)	78 ± 11	79 ± 10	0.2895
Fasting plasma glucose (mmol/L)	4.97 ± 0.44	4.97 ± 0.44	0.8317
OGTT 2-h plasma glucose (mmol/L)	6.13 ± 1.66	6.21 ± 1.59	0.3941
HbA1c (mmol/mol)	38 ± 4.4	38 ± 4.4	0.5894
HbA1c (%)	5.6 ± 0.4	5.6 ± 0.4	0.5894
HOMA2-IR	0.77 (0.48-1.13)	0.78 (0.51-1.14)	0.4342
HOMA2%B	81.15 (61.1-103.7)	82 (63.1-105)	0.5205
Total cholesterol (mmol/L)	5.02 ± 0.91	5.01 ± 0.93	0.9075
Triglyceride (mmol/L)	1.02 (0.72-1.46)	1.00 (0.72-1.50)	0.9330
HDL cholesterol (mmol/L)	1.32 ± 0.33	1.35 ± 0.33	0.1135
LDL cholesterol (mmol/L)	3.05 ± 0.82	3.02 ± 0.83	0.5919
Uric acid (μmol/L)	330 ± 89	325 ± 87	0.4162
hsCRP (nmol/L)	0.86 (0.38-1.71)	0.76 (0.38-1.52)	0.7063

Means ± SDs or medians (interquartile ranges) are shown.

Abbreviations: OGTT, oral glucose tolerance test; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitive C-reactive protein

科技部補助計畫衍生研發成果推廣資料表

日期:2016/09/04

科技部補助計畫	計畫名稱: 慢性感染與糖尿病和代謝症候群之世代研究
	計畫主持人: 魏榮男
	計畫編號: 104-2314-B-041-002- 學門領域: 公衛及環境醫學

無研發成果推廣資料

104年度專題研究計畫成果彙整表

計畫主持人：魏榮男	**計畫編號：**104-2314-B-041-002-
計畫名稱：慢性感染與糖尿病和代謝症候群之世代研究	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			1	篇	投稿中
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		

	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	1	人次	嘉南藥理大學蕭琦真
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	2		廖瑩竹工作8個月，之後許心華接任2個月
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：□已發表　■未發表之文稿　□撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   本研究發現胃萎縮對於糖尿病發生反而是個保護因子；然而，幽門螺旋桿菌的感染就與糖尿病的發生卻沒有統計相關（$p>0.05$）。本研究發現是目前文獻的第一篇，究竟胃萎縮透過甚麼樣機轉而降低糖尿病的發生，目前尚未釐清，我們猜測，是否與胃分泌賀爾蒙ghrelin的缺乏有關，ghrelin控制了食慾、能量代謝與體內葡萄糖的平衡，當其濃度較高時，會刺激食物攝取增加，導致肥胖，並刺激生長素分泌與降低，降低胰島素的分泌並導致高血糖。此外，本世代研究也發現B、C 型肝炎感染與糖尿病發生也沒有相關。

   本研究主要貢獻有以下幾點：
   (1)有助釐清從健康進展到糖尿病的自然史，此為防治糖尿病最基本的理論依據之一。
   (2)國內B、C肝炎帶原高達400萬人左右，其對於糖尿病影響的研究，亟需釐清，本研究提供了重要的參考數據。
   (3)探討幽門螺旋桿菌與代謝症候群的關係，是否導致胃萎縮之後，才開始影響糖尿病的發生，這需要未來研究更進一步深入探討。
   (4)關於糖尿病的發生越多了解，對於糖尿病的防治，將越能有效。

4. 主要發現

本研究具有政策應用參考價值：■否　□是，建議提供機關
（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
本研究具影響公共利益之重大發現：■否　□是
說明：（以150字為限）