(*H. pylori* colonization and risk of diabetes: an ancillary analysis in the Diabetes Prevention Program)

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(Saud Alzahrani)

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Advisors:

Anastassios Pittas, MD, MS

Jessica Paulus, ScD

Robin Ruthazer, MPH

Jason Nelson, MS

ABSTRACT

Recently, there are conflicting reports on the association between *H. pylori* infection and clinical manifestations of the metabolic syndrome, we aimed to prospectively investigate the association between *H. pylori* infection and risk of incident diabetes in adults at high risk for diabetes who participated in the Diabetes Prevention Program (DPP) study. In a nested case-control study conducted among 421 adults with newly diagnosed diabetes and 421 matched controls in the DPP study, we examined the association between serostatus of *H. pylori* at baseline (collected when all participants were free of diabetes) and risk of incident diabetes over a mean follow-up period of 2.6 years. We also examined the cross-sectional association between *H. pylori* infection and insulin sensitivity, insulin secretion and the oral disposition index at baseline. Analyses were adjusted for matching variables (age, sex, race, DPP intervention arm and length of follow up) and potential confounders. At baseline, *H. pylori* infection was present in 40% of cases and 39% of controls. After adjusting for matching factors, there was no association between *H. pylori* infection and incident diabetes (odds ratio [OR] of 1.04 (95% CI, 0.77 to 1.40). Further adjustment for body mass index at baseline did not change the relationship (OR 1.04; 95% CI, 0.76 to 1.42). *H. pylori* infection was negatively associated with insulin sensitivity index and disposition index, and positively associated with insulin secretion, but these findings were not statistically significant. In adults at high risk for diabetes, *H. pylori* infection was not associated with of incident diabetes. Insulin sensitivity, insulin secretion and oral disposition index at baseline did not significantly differ between participants with *H. pylori* infection or without.

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DPP: Diabetes Prevention Program

DI: Disposition Index

BMI: Body mass index

IgG: Immunoglobulin G

ISR: Immune status ratio

OD: Optical Density

CIR: Corrected insulin response

ISI: Insulin-sensitivity index

INTRODUCTION

The incidence of diabetes is increasing at an alarming rate both nationally and worldwide with 1.9 million new cases diagnosed in 2010 in the U.S. alone.[1] Nearly 9 out of 10 new cases are due to type 2 diabetes. In clinical trials, lifestyle changes have been successful at lowering risk of the disease. In the Diabetes Prevention Program (DPP) study, among adults at high risk for diabetes, intensive lifestyle intervention reduced the risk of incident diabetes by 58%[2]. However, long-term weight-maintenance in the clinical setting has proved elusive. Moreover, even after successful weight loss, there is still significant residual risk.

Lifestyle (e.g., diet, physical activity), genetic, and socioeconomic factors are among the well-established risk factors for diabetes.[3,4] Recently, there are conflicting reports on the association between *H. pylori* infection and clinical manifestations of the metabolic syndrome. Whereas *H. pylori* was initially thought only to cause disease in the upper gut [5,6], this microorganism has been associated with several extra-digestive conditions[7], including type 2 diabetes. Epidemiologic studies to date have reported inconsistent associations between *H. pylori* infection and diabetes risk.[8-25] However, all of these studies, with the exception of one,[26] have been cross-sectional designs, which cannot determine the temporal direction of the association between infection and diabetes status. Furthermore, the observed associations may be confounded by a variety of factors (e.g. age, non-white race). Another common methodological limitation observed in many of these studies was reliance on self-reports to identify diabetes cases, which may result in significant misclassification. Lastly, while several studies have reported the association between *H. pylori* infection and insulin resistance;[12,21,22,27,28] none have examined the association between *H. pylori* infection and composite beta-cell function as measured by disposition index, which predicts development of diabetes.[29]

Using data from a large multi-racial cohort of U.S. adults with pre-diabetes who participated in the DPP study, a multicenter randomized controlled trial comparing different treatment modalities to prevent incident diabetes, in which strict biochemical glycemic criteria followed to define pre-diabetes and diabetes, we prospectively examined the association between infection with *H. pylori* and development of diabetes, using a nested case-control study design. In cross-sectional analyses, we examined the association between *H. pylori* infection and insulin sensitivity, insulin secretion and oral disposition index.

RESEARCH DESIGN AND METHODS

2.1 Study Participants

The DPP was a randomized controlled clinical trial conducted between 1996 and 2001 at 27 sites in the U.S., comparing the effects of intensive lifestyle intervention, metformin, or placebo on the development of diabetes in adults at high risk for the disease[2]. The eligibility criteria, design, and methods of the DPP have been described in detail elsewhere.[2,28] Inclusion criteria included age ≥25 years, body mass index (BMI) ≥24 kg/m^2 (≥22 kg/m^2 in Asian Americans), fasting plasma glucose between 5.3 to 6.9 mmol/L (95 to 125 mg/dL) (≤6.9 mmol/L for American Indian sites) and plasma glucose between 7.8 to 11 mmol/L (140 to 199 mg/dL) after a 75-gram oral glucose tolerance test. The primary exclusion was any medication known to alter glucose tolerance. The Institutional Review Board at each site approved the DPP protocol and all participants gave written informed consent. The Tufts University Institutional Review Board approved the present ancillary observational study.

All participants were given standard advice on healthy diet and physical activity before randomization to one of 3 arms: intensive program of lifestyle modification (aiming to achieve a

weight reduction of at least 7 percent of initial body weight), standard lifestyle recommendations plus metformin or standard lifestyle recommendations plus placebo. The present observational study was conducted in a cohort selected from the participants randomized to two arms, intensive lifestyle (n=1,017) and placebo (standard lifestyle, n=1,023)

We planned to test our hypothesis and accomplish the goal of this stud by pursuing the following Specific Aims in a prospective observational study utilizing resources within the Diabetes Prevention Program (DPP) study, which is trial comparing different treatment modalities to prevent incident diabetes in people at risk for diabetes. This study involved: (1) use of stored specimens to generate new data and (2) used of data that already exist in the DDP database.

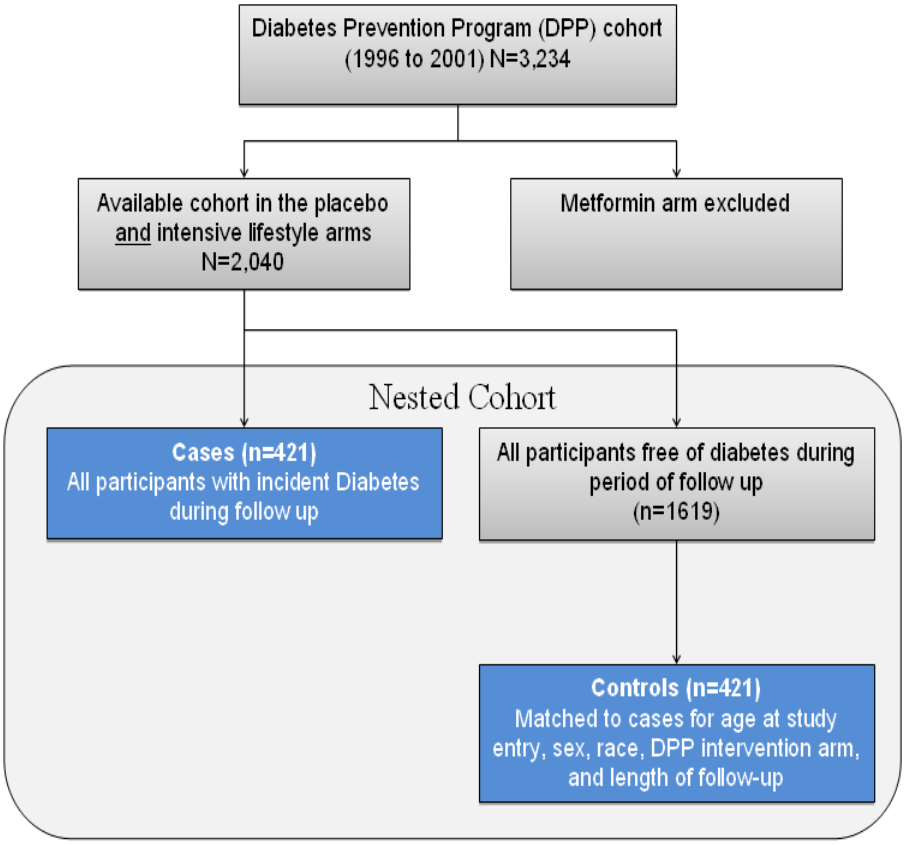
Aim 1 - To determine the association between *H. pylori* infection and incident diabetes in a case-control study design, nested within the life style and placebo arms of DPP study population. The working hypothesis is that *H. pylori* positive status, as measured by serum *H. pylori* immunoglobulin G (IgG) antibody level, is associated with progression to clinical diabetes, after multivariable adjustment.

Aim 2 - To determine the association between *H. pylori* infection and insulin sensitivity, insulin secretion and the oral disposition index at baseline, in a cross-sectional study design using data obtained from the baseline visit of the DPP. The working hypothesis is that *H. pylori* positive status, as defined above, is associated with impaired insulin sensitivity, insulin secretion and the oral disposition index compared to *H. pylori* negative status participants, after multivariate adjustment.

2.2 Nested Case-Control Design

Cases were DPP participants in the lifestyle and placebo arms who developed diabetes during follow-up (n=421). Controls were selected from the DPP cohort of non-cases and matched 1:1 with cases using the "greedy" algorithm.[30] Case-control matching criteria was based on age at study entry (+/- 5 years), sex (male or female), race (White vs. Non-White), DPP intervention (lifestyle vs. placebo), and length of time in the DPP study prior to diabetes diagnosis (for cases) and last visit (for controls). Cases and controls were only matched if the control was enrolled for an equal or longer time than the case it was matched with. Cases and controls had a mean follow up of 2.6 years (1.8 and 3.3 years for cases and controls, respectively) to monitor for the outcome of interest, incident diabetes (Figure 1).

Figure 1 Nested case-control design



2.3 Measurement of H. pylori

Testing for *H. pylori* infection was done in stored samples from the baseline visit, when all participants were free of diabetes, by measuring *H. pylori* immunoglobulin G (IgG) antibody in serum using *H. pylori* IgG enzyme-linked immunosorbent assay (ELISA, Diasorin Diagnostics Srl-Manufactured by Hycor Biomedical GmbH). The clinical laboratory at Tufts Medical Center performed the testing and all quality control procedures were followed. Samples from matched case-control pairs were assayed in the same analytical run by personnel blinded to the case-control status of the samples. For each specimen, an immune status ratio (ISR) was calculated based on Optical density (OD) values. A value of ISR less than 0.9 is considered to be negative, ISR between 0.91-1.09 is considered indeterminate and ISR greater than 1.1 was considered positive. In sensitivity analysis, we did not find a significant change when indeterminate samples (27 samples) were grouped with either negative or positive samples, so we considered all samples with ISR of 0.91 or greater as positive as done in prior studies.[17]

2.4 Ascertainment of Incident Diabetes

The primary DPP outcome, development of diabetes, was assessed following strict laboratory criteria, based on oral glucose (75-gram) tolerance testing performed annually and fasting plasma glucose performed semiannually or when symptoms consistent with hyperglycemia occurred. The diagnosis required confirmation by repeat testing.

2.5 Ascertainment of Glucose-Insulin Dynamic Measures

A composite beta-cell function (oral disposition index) was estimated by the product of the corrected insulin response (CIR) and insulin-sensitivity index (ISI), as previously used in the DPP.[29] Corrected insulin response was calculated as follows: (100 × insulin at 30 minutes) ÷ (glucose at 30 minutes × [glucose at 30 minutes – 70 mg/dL]). Insulin-sensitivity index, which is the reciprocal of insulin resistance according to the homeostasis model assessment, was calculated as follows: 22.5 ÷ (fasting insulin × [fasting glucose ÷ 18.01]). Glucose and insulin are expressed as mg/dL and μU/mL, respectively, unless otherwise specified.

2.6 Assessment of Potential Confounders and Laboratory Assessment

Self-reported race/ethnicity was classified according to the 1990 U.S. Census questionnaire. Self-reported level of leisure physical activity was assessed annually with the Modifiable Activity Questionnaire and expressed as the average metabolic equivalent (MET-hours) per week for the previous year.[2] Standardized interviewer-administered questionnaires were used annually to obtain self-reported data on personal medical history, smoking, medications, alcohol use, and family medical history. Weight was measured using a standard calibrated scale and height was measured with a standard stadiometer and body mass index was calculated (Kg/m^2). Hypertension was defined as blood pressure higher than 140/90 mmHg or use of antihypertensive medication. Fasting blood was obtained and processed following standardized procedures.

2.7 Statistical Analyses

We describe the differences in baseline characteristics between cases and controls using means for continuous measures and percentages for categorical measures. Statistical tests of the mean differences between cases and controls were performed with t-tests and chi-square tests for proportional differences. On the basis of *H. pylori* immunoassay results, as described above, participants were classified as *H. pylori* positive or negative. We estimated odds ratios (OR) to measure the association between *H. pylori* status and incident diabetes using conditional logistic regression analysis. We used matched pairs as strata and adjusted for the following baseline characteristics: BMI (kg/m^2), smoking status at baseline (never, past, or currently smoking), alcohol consumption (g/day) and physical activity (MET-hours per week). Selecting of the covariates in the model was based on clinical acumen. Smoking status and alcohol consumption were included because they are established risk factors for both *H. pylori* infection and diabetes. Additional variables considered for the multivariate model included BMI and physical activity at baseline because they are related to diabetes and adjusting for them could improve the precision of the model. The first conditional logistic regression model was unadjusted for other characteristics. Two additional regression models were built forcing in successive baseline measures. The first additional model included baseline BMI. Then a second model was built adjusting for BMI, smoking status, alcohol consumption and physical activity.

In subgroup analyses, we tested the association between *H. pylori* and incident diabetes separately for each group, by race (White and Non-White), BMI (non-obese vs. obese [<30 and >=30 kg/m^2]), age (two groups based on median [<57 and >=57 years]) and DPP intervention (lifestyle and placebo). We tested also for statistical interaction between race, baseline BMI, age, and DPP intervention x *H. pylori* status on diabetes incidence by including an interaction term in the conditional logistic regression model.

In cross-sectional analyses, we assessed the association between *H. pylori* status and glucose-insulin dynamic measures. Average differences of insulin sensitivity index, insulin secretion and disposition index at baseline between *H. pylori* groups was calculated from multivariate linear regression models adjusting for the same variables above, excluding diabetes status.

For all analyses a p-value of 0.05 was used as the threshold for determining statistical significance. Analyses were performed using SAS (version 9.3).

RESULTS

3.1 Participant characteristics

At baseline, the mean age of the cohort was 49.6 years and 66% were female (Table 1). Participants who developed diabetes were more likely to be smokers and reported lower alcohol consumption. As expected, cases had a higher BMI (35.6 vs. 33.2 kg/m^2), Hemoglobin A1c (6.1 vs. control 5.8%), fasting plasma glucose (112.1 vs.104.9 mg/dL) and CRP (6.8 vs. 5.5 mg/L) as compared to controls (Table 1).

Table 1. Baseline characteristics of cases and control participants

Characteristic	Diabetes cases (n=421)	Controls (n=421)	P-value[1]
Age, mean (SD), y	49.6 (10.0)	49.6 (9.9)	0.96
Sex, No. (%) women	278 (66.0)	278 (66.0)	1.0
Race, No. (%)			
White	228 (54.2)	228 (54.2)	0.22
African-American	95 (22.6)	78 (18.5)	
Other (Hispanic, Asian, American Indian)	98 (23.3)	115 (27.3)	
Weight, mean (SD), kg	99.8 (22.8)	91.9 (18.9)	<0.01
DPP lifestyle intervention arm, No (%)	139 (33.0)	139 (33.0)	--
Body mass index, mean (SD), kg/m^2	35.6 (7.5)	33.2 (6.1)	<0.01
Waist circumference, mean (SD), cm	109.4 (16.1)	103.2 (13.8)	<0.01
Hypertension, No. (%) [3]	121 (28.7)	112 (26.6)	0.49
Family history of diabetes, No. (%)	281 (66.9)	300 (71.3)	0.17
Physical Activity, mean (SD), MET-hours [4]	16.6 (23.2)	15.1 (19.7)	0.33
Smoking status, No. (%)			
Never	230 (54.6)	257 (61.0)	0.01
Past	150 (35.6)	144 (34.2)	
Current	41 (9.7)	20 (4.8)	
Alcohol consumption, mean (SD), g/day	1.8 (4.6)	2.3 (5.8)	0.19
Systolic blood pressure, mean (SD), mmHg	125.1 (14.1)	121.8 (14.2)	<0.01
Diastolic blood pressure, mean (SD), mmHg	78.6 (9.8)	78.1 (9.4)	0.43
Fasting plasma glucose, mean (SD), mg/dL	112.1 (9.4)	104.9 (6.3)	<0.01
Hemoglobin A1c, mean (SD), %	6.1 (0.6)	5.8 (0.5)	<0.01
Insulin sensitivity index (ISI), mean (SD), [(μU/mL)*(mg/dL)]$^{-1}$	0.16 (0.12)	0.21 (0.14)	<0.01
Corrected insulin response (CIR), mean (SD), [(μU/mL)/(mg/dL)^2]	0.53 (0.34)	0.66 (0.41)	<0.01
Disposition Index (LN(CIR) * LN(Insulin sensitivity index))	0.07 (0.04)	0.12 (0.08)	<0.01
C-reactive protein, mean (SD), mg/L	6.8 (9.3)	5.5 (6.2)	0.02
H. pylori positive, N (%)	169 (40%)	166 (39%)	

[1] Case and control participants' characteristics were compared using the *t* test (for means) or chi-square test (for percentages). Age (+/- 5 years), sex (men or women), race (White vs. Non-White), DPP intervention arm (lifestyle or placebo) and length of follow up were matching variables.

[3] Hypertension defined as blood pressure ≥130/85 mmHg or the use of antihypertensive medication. [4] MET denotes metabolic equivalent. MET-hours represent the average amount of time engaged in specified physical activities multiplied by the MET value of each activity. SD, standard deviation;

3.2 H. pylori infection and incident diabetes

Participants were followed for an average of 2.6 years. After adjusting for matching factors, *H. pylori* infection was not significantly associated with increased risk of type 2 diabetes (odds ratio [OR] of 1.04 (95% CI, 0.77 to 1.40) (**Table 2**). After further adjustment for BMI at baseline, there was no change in the association (OR 1.04; 95% CI, 0.76 to1.42). Further multivariate adjustment for other covariates at baseline, including smoking status, alcohol consumption and self-reported physical activity also did not change the magnitude of the association between *H. pylori* status and incident of diabetes.

Table 2. *H. pylori* status and incident diabetes in DPP participants with pre-diabetes

	***H. pylori* status** [*]		**P-value**
	Negative (N=507)	**Positive (N=335)**	
Cases / controls, No.	252 / 255	169 / 166	
Model 1 [1]	1.00 (reference)	1.04 (0.77, 1.40)	0.82
Multivariate plus Body Mass Index [2]	1.00 (reference)	1.04 (0.76, 1.42)	0.79
Multivariate model [3]	1.00 (reference)	1.03 (0.74, 1.42)	0.88

[1] Model 1 adjusted for matching factors (age [years], sex [male or female], race [White vs. Non-White], intervention arm [lifestyle or placebo] and length of follow up) through conditional logistic regression.
[2] Model 2 adjusted for everything in model 1 plus body mass index (kg/m^2) at baseline.
[3] Model 3 adjusted for everything in model 2 plus smoking status (never, past, or currently smoking), alcohol consumption (g/day), self-reported physical activity (MET-hours per week).

3.3 Subgroup Analyses

We conducted subgroup analyses, to explore whether race, baseline BMI, age or DPP intervention would modify the association between *H. pylori* infection and incident of diabetes (**Table 3**). The test for interaction was nearly statistically significant for *H. pylori* x BMI (p= 0.0833), but the association between *H. pylori* infection and incident diabetes was not significant within the two BMI subgroups. The tests for interactions were not statistically significant in any of the other strata analyzed.

Table 3. *H. pylori* status and incident diabetes, by race, baseline body mass index, age and DPP intervention arm.

	H. pylori status		**P-value**[1]	**P for interaction**[6]
	Negative	**Positive**		
Race [2]				
White	1.00 (reference)	1.22 (0.81, 1.86)	0.35	0.25
Not-White	1.00 (reference)	0.88 (0.59, 1.32)	0.53	
Body Mass Index (kg/m^2) [3]				
BMI < 30	1.00 (reference)	1.10 (0.66, 1.83)	0.73	0.08
BMI ≥ 30	1.00 (reference)	1.03 (0.72, 1.47)	0.87	
Age (median, years) [4]				
< 57	1.00 (reference)	1.00 (0.71, 1.41)	0.99	0.84
≥ 57	1.00 (reference)	1.14 (0.64, 2.02)	0.65	
DPP Arm				
Lifestyle	1.00 (reference)	1.00 (0.59, 1.68)	0.99	0.87
Placebo	1.00 (reference)	1.05 (0.74, 1.49)	0.79	

[1] P-values for subgroup analyses are based on logistic regression adjusted for matching factors (age [years], sex [male or female], race [White vs. Non-White], DPP intervention arm [lifestyle or placebo]) and length of follow up.
[2] Model for White includes 228 cases and controls; model for non-White includes 193 cases and controls.
[3] Model for BMI<30 kg/m^2 includes 110 cases and 149 controls; model for BMI≥30 kg/m^2 includes 311 cases and 272 controls
[4] Model for age<57 years includes 318 cases and 316 controls; model for age≥57 years includes 103 cases and 105 controls
[5] Model for DPP lifestyle arm includes 139 cases and controls; model for placebo arm includes 282 cases and controls.
[6] P-values for interaction effect are adjusted for matched variables (age [years], sex [male or female], race [White vs. Non-White], intervention arm [lifestyle or placebo]) and length of follow up through conditional logistic regression.

3.4 H. pylori infection and Glucose-Insulin Dynamic Measures

We also examined the cross-sectional association between *H. pylori* infection and insulin sensitivity index, corrected insulin response and disposition index at baseline adjusting for matching factors and other covariates at baseline including smoking status, alcohol consumption and self-reported physical activity. *H. pylori* infection was negatively associated with insulin sensitivity index and disposition index and positively associated with corrected insulin response, but these associations were not statistically significant (**Table 4**).

Table 4. Adjusted average difference of insulin secretion and insulin sensitivity according to *H. pylori* status at baseline in the lifestyle and placebo arms of the Diabetes Prevention Program

	H. pylori status *		P-value
	Negative	Positive	
†Insulin sensitivity index, [(μU/mL)*(mg/dL)]-1	(reference)	-0.02 (-0.10, 0.05)	0.53
†Corrected insulin response, CIR (μU/mL)/(mg/dL)2	(reference)	0.08 (-0.01, 0.17)	0.08
Disposition Index (LN(CIR) * LN(Insulin sensitivity index))	(reference)	-0.12 (-0.29, 0.05)	0.17

Results are presented by *H. pylori* status; average difference between groups was calculated from the multivariate linear regression model; all models are adjusted for matching factors (age [years], sex [male or female], race [White vs. Non-White], DPP intervention arm [lifestyle or placebo] and length of follow up), BMI at baseline, smoking status (never, past, or currently smoking), alcohol consumption (g/day), self-reported physical activity (MET-hours per week).
†Dependent variable log transformed

DISCUSSION

In this prospective observational study nested within the DPP cohort, there was no association between *H. pylori* infection and incident diabetes even after adjustment for potential confounders and risk factors. No associations were observed in subgroups defined by race, baseline BMI, age or DPP intervention arm. Also, there was no statistically significant cross-sectional association between *H. pylori* infection and measures of glucose-insulin dynamics at baseline.

The prevalence of *H. pylori* infection in patients with diabetes and its relation with glycemic control has been studied with discordant results (table 5). Some authors have found high prevalence of the infection in such patients and infection having an adverse effect on metabolic control[8-11,14,23]. Potential biological mechanisms that might explain these findings include the role of *H. pylori* in energy homeostasis by affecting the production of ghrelin and leptin, which are important hormones in the regulation of appetite and energy expenditure.[31-33] Another potential mechanism is through an effect on secretion of pro-inflammatory cytokines, known to influence the metabolic syndrome[34].

Other studies have reported no differences in *H. pylori* between diabetic and non-diabetic populations, or even a lower rate of infection in diabetic patients.[18-20,24,25,35] In such cases, the results may potentially be explained by the higher exposure to antibiotics taken by patients with diabetes (particularly by older participants) resulting in more frequent occasional clearance of the infection.

The use of a case-control study nested within a well-conducted randomized trial has several advantages over prior studies. First, cases were defined based on a new diagnosis of diabetes (incident cases only) using strict biochemical glycemic criteria to define diabetes, which avoids

misclassification associated with self-reporting of diabetes status. Second, the assessment of diabetes risk factors and other potential confounders was carried out prospectively before diabetes development, and was considerably more detailed than in the earlier studies. Third, this study examined the association between *H. pylori* infection and the oral disposition index, which is a measure of pancreatic beta cell function that captures the hyperbolic relation between insulin secretion and insulin sensitivity. A low disposition index indicates an impaired pancreatic beta cell function and is a validated predictor of diabetes risk.[29,36] The study is limited by the lack of data on the use of antibiotics and proton pump inhibitor during the follow up period, which could alter the course of *H. pylori* infection. Participants in our study had higher mean HbA1c at baseline compared to mean HbA1c in participants NHANES III, where *H. pylori* infection was positively correlated with HbA1c[17]. It is possible that an actual effect of *H. pylori* on insulin sensitivity and beta-cell function may occur before the onset of pre-diabetes. Another potential limitation is that the cross-sectional analysis was conducted among those participants matched on diabetes risk factors in the case-control design, and thus the results may be less generalizable to all patients with pre-diabetes.

In conclusion, we did not find an association between *H. pylori* infection and diabetes incidence in participants at high risk of diabetes. Insulin sensitivity, insulin secretion and oral disposition index at baseline did not significantly differ between participants with *H. pylori* or without. Further studies with longer duration between *H. pylori* infection, particularly with the cytotoxic strain, and development of diabetes are warranted.

Table 5: Reviewed Observational studies of *H. pylori* status and diabetes

Study, Year (reference) Cohort [Country]	Male, %	Mean baseline age (range), y	White, %	n/N (incidence)	*H. pylori* measure	Outcome (ascertainment method)	Results, Prevalence, OR, or HR (95% CI) P for trend	Is there Association?	Adjustments	Study Quality comments
					Cross-sectional					
(Oldenburg, Diepersloot et al. 1996) [Netherlands]	NA	DM insulin requiring : 38.6 DM non-insulin requring : 66.2	NA	N for DM insulin requiring: 45 N for DM non-insulin requiring: 98 Control: 159	IgG IgA	? self-report	*HP+ Prevalence by IgG* *75% in DM- non insulin requring vs. 35% in controls at age 60-70* *(p<0.05)* *HP+ Prevalence 67% in DM- insulin requring vs. 24% in controls at age 50-60* *(p<0.05)*	Yes	Not done	poor
(de Luis, de la Calle et al. 1998) [Spain]	50%	For DM: 24 (16–55)	NA	N for DM: 80 Control: 100	IgG	DM insulin requiring only (Based use of Insulin)	*HP+ Prevalence 47% in DM vs. 33% in controls (p=NS)*	No	Not Done	Included both T1DM And T2DM
(Gentile, Turco et al. 1998) [Italy]	52%	Cases: 45–61 Control: 44–63		N: 325	UBT histology	DM based on WHO guidelines including OGTT (used in control)	Prevalence 74 vs. 50% In DM vs. non-DM p <0.01	Yes	Not done	Fair
(Begue, Mirza et al. 1999) [US]	36 (51%)	11(2–18)	36	T1DM NA/71 (NA) 11 (15.5%) were found to be infected. *H pylori* infection	IgG UBT	Insulin daily requirement (IU/Kg/day) HbA1c (capillary electrophoresis system)	Insulin requirement increase by ~0.33 IU/kg/d 3% increase in HbA1c	Yes (with insulin requirement / Hba1c level)	Age, sex, race, Duration of illness, income, compliance	Fair
(Dore, Bilotta et al. 2000) [Italy]	NA	>40	NA	/240	*IgG*	Diabetes (self-reported)	*HP+ Prevalence 62% in T2DM vs. 63% in controls (p=NS)*	No	Age, socioeconomic class	Fair 18% of H. pylori + were confirmed by UBT.

(Xia, Talley et al. 2001) [Australia]				T1DM: 49 T2DM: 380 Control:170	*IgG*	Diabetes? self-report	*HP+ Prevalence 33% in DM vs. 32% in controls (p: ns)*	No	Not done	T1DM and T2DM added together
(Marrollo, Latella et al. 2001) [Italy]	42% for DM	63 for DM	NA	DM = 74 control=117	UBT histology	DM type 1 and typ2 (based on ADA criteria 1998)	*HP+ Prevalence 65% in DM vs. 48% in controls (p< 0.05)*	Yes	Not done	T1DM and T2DM added together poor
(Anastasios, Goritsas et al. 2002) [Greece]	NA	56	NA	67 diabetics and 105 control	Histology	DM based on self-report and confirmed by FBG > 126 mg/dl	*HP+ Prevalence 37.3% in DM vs. 35.2% in controls (p= 0.78)*	No	Not Done	
(Candelli, Rigante et al. 2003) [Italy]	66 (55%) T1DM	14.8 +/- 5.6 (6–21)	?	T1DM NA/121 T1DM NA/ 147 control	UBT Cag-A	T1DM	odds ratio:0.98 (0.58–1.66) *P value 0.*954	No	Age, sex, social class	
(Nabipour, Vahdat et al. 2006) [Iran]	49.2%	36.1% (25–34) 29.0% (35–44) 21.9% (45–54) 12.7% (55–66)	N/A	NA/ 1791 52.1% with metabolic syndrome	*IgG*	Metabolic syndrome by NCEP-ATP III criteria including fasting serum glucose >= 6.1 mmol/l,	HP+ [OR = 1.50 (1.12–2.00); p =0.007] in men HP+ [OR = 1.45 (1.09–1.94); 0.01] In Women	Yes	?	
(Bener, Micallef et al. 2007) [Turkey]	?	47.2 +/- 6.8 in DM with High IgG	NA	N for DM: 210 N for control: 210	*IgG* *IgA*	DM based on ADA criteria or use of diabetic medications	*HP+ Prevalence in DM vs. Control* 63.3% vs 48.1% for IgA>250 titer and 76.7% vs 64.8% for IgG>300 titer P is not reported	Yes	Not done	poor
(Longo-Mbenza, Nkondi Nsenga et al. 2007) [Congo]	130 (63.4 %)	53.1±14.4	N/A	205 62.4% (tested positive for the H. pylori antibody)	*IgG*	DM Based on Fasting blood glucose	FPG 104.5±40.8 in H. pylori+ vs. 96.7±44.6 H. Pylori–	Yes		Good
(Aydemir, Bayraktarogl	HP+ (15)	HP+ (46.1 ±		HP + (36)	Histology		HOMA-IR	Yes (with	Age, sex, BMI	

u et al. 2005)	HP- (13)	10.1) HP- (48.5 ± 10.7)		HP – (27)			HP+ (2.56 ± 1.54) HP- (1.73 ± 1.10)	IR)		
(Aslan, Horoz et al. 2006) [Turkey]	HP+ (20) HP- (26)	HP+ (37 ± 12) HP- (35 ± 15)		Dyspepsia HP+ (48) HP- (55)	UBT histology	HOMA-IR	HOMA-IR HP + 1.67 ± 0.99 HP- 0.89 ± 0.47	Yes (with IR)		Fair
(Ozdem, Akcam et al. 2007) [Turkey]	HP+ (10) HP- (11)	HP+ 12 (6–17) HP- 13 (5–16)		Dyspeptic children and adolescents HP+ (20) HP- (31)	*Histology*	HOMA-IR	HOMA-IR HP+ (1.41 (0.60–4.87) HP- (0.91 (0.13–2.43)	Yes (with IR)		
(Demir, Gokturk et al. 2008) [Turkey]				141 cases (t2DM);142 control participants	*UBT Histology*	DM (self-report) HbA1c Fasting serum glucose	prevalence of H. pylori 61.7% vs 58.5%, among type 2 diabetic Vs nondiabetic controls P = 0.577 HbA1c, Fasting glucose not differs between both groups.	No		
(Fernandini-Paredes, Mezones-Holguin et al. 2008) [Venezuela]	46%	adult	NA	Total N= 75 65.3% with HP+	*UBT Histology*	DM with HbA1c > 7 Vs. DM with a1c < 7	OR, 1.67; CI 95%, 0.56– 5.03 *P*= 0.434	No		
(Gunji, Matsuhashi et al. 2008) [Japan]	74%	Adult	NA	Total N: 7,394	*IgG*	Metabolic syndrome (including FBS > 110 mg/dL)	OR 1.39, 95% CI 1.18-1.62	Yes		
(Gunji, Matsuhashi et al. 2009) [Japan]	IR+ (96) IR- (892)	IR+ (48.6 ± 8.7) IR- (49.6 ± 8.6)		IR+ 99 IR- 1008	IgG	HOMA-IR	I HOMA-IR IR+ (3.51 ± 1.13) HP+ (39.4 %) IR- (1.16 ± 0.47) HP+ (28.7%)	Yes (with IR)		
(Eshraghian, Hashemi et al. 2009) [Iran]	HP+ (21) HP- (13)	HP+ (32.2 ± 14.2) HP- (33.0 ± 8.1)		Healthy HP+ (43) HP- (28)	*IgG*	HOMA-IR	HOMA-IR HP+ (3.54 ± 2.20) HP- (2.46 ± 1.90)	Yes (with IR)		
Gao et al 2009 [China]	HP+ (26) HP- (25)	HP+ (45.9 ± 2.4) HP-		Healthy HP+ (50) HP- (50)	*Histology IgG (Both tests needed to be positive)*	HOMA-IR	HOMA-IR HP+ (1.6 ± 0.2) HP- (1.7 ± 0.3)			

		(43.3 ± 2.9)								
(Lutsey, Pankow et al. 2009) [US]	?	(45–84)	?	1000 H. pylori seropositive (45.4%)	IgG	Diabetes based on FBS or use of DM medication	Prevalence ratio of diabetes by H. Pylori Crude 1.65 (1.16, 2.34) Race/ethnicity-adjusted 1.21 (0.84, 1.74) Demographic-adjusted 1.12 (0.78, 1.62) (No association after adjustment)	No	age , sex, race/ethnicity, education, site	
Chen and Blaser NHANES III (Chen and Blaser 2012) [US]	50%	00-75	30	NA/6072 (NA);	*IgG*	Diabetes (self-reported based on physician-diagnosed or insulin use) HbA1c	There was no association between H. pylori and history of self-reported diabetes -HP+ associated with higher mean HbA1c (P < .01))	No (with DM) Yes (with HbA1c level)	Age, sex, BMI, SES	Good
(Jafarzadeh, Rezayati et al. 2013) IRAN	T2DM	Adult		100 patients with type 2 diabetes and 100 age-matched healthy individuals	*IgG* *Cag-A*	DM based on fasting serum glucose	No association	No		Poor?
(Hsieh, Wang et al. 2013) CHINA	T2DM	Adult		N= 2070	*IgG*	HbA1c HOMA-B	HP+ vs. HP- HbA1c mean in 5.78% vs. 5.69%, P = 0.01 HOMA-B level 53.85 +/- 38.43 vs. 60.64 +/- 43.40, P = 0.009	Yes (withHbA1c)		
(Vafaeimanesh, Heidari et al. 2014) IRAN	T2DM	Adult		211 patients already have T2DM but not on Insulin	*IgG*	Adiponectin HOMA-IR	Change in Adiponectin not statistically significant IR (HP- = 3.160 ± 3.327 versus HP+ = 4.484 ± 3.781, P = 0.013)	No (with adiponectin) Yes (with IR)		Poor?
					Longitudinal					

Sacramento Area Latino Study on Aging (Jeon, Haan et al. 2012) [US]	39	60-101	0	144/782 (-18.4)	*IgG*	Type 2 diabetes (self-report, FPG≥126 mg/dL, or medication-treated,	HR: 2.69 (95% CI 1.10, 6.60)	Yes	Age, sex, BMI? education, cardiovascular disease, smoking, cholesterol, IR, CRP, IL-6	Good

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