Differences in glycated hemoglobin levels and cholesterol levels in individuals with diabetes according to *Helicobacter pylori* infection

Saeda Haj¹, Gabriel Chodick¹,², Sophy Goren¹, Wasef Na’amnih¹, Varda Shalev¹,² & Khitam Muhsen¹*

This study examined differences in glycated hemoglobin (HbA1c), fasting plasma glucose and cholesterol levels between *H. pylori* infected and uninfected persons with diabetes. Anonymized data of Maccabi Healthcare Services in Israel were analyzed, of 12,207 individuals (50.0% *H. pylori* positive) aged 25–95 years who underwent the urea breath test. The data included HbA1c, fasting plasma glucose and cholesterol levels. The inverse probability of treatment weighting approach was used to account for confounders. Differences between individuals who were *H. pylori* positive and negative, in HbA1c (> or ≤ 7.0%) and in cholesterol levels were assessed using weighted generalized estimating equations. For men, but not women, the likelihood of having HbA1c > 7.0% was increased in those infected than uninfected with *H. pylori*: prevalence ratio 1.11 (95% CI 1.00, 1.24), *P* = 0.04. For both sexes, total cholesterol (*P* = 0.004) and low-density lipoprotein (LDL) levels (*P* = 0.006) were higher among those infected than uninfected with *H. pylori*. No significant differences were found in glucose and HDL levels according to *H. pylori* infection. The results were consistent in unweighted multivariable analyses. In conclusion, *H. pylori* infection might be related to worse glycemic control in men, and higher total cholesterol and LDL cholesterol levels in both sexes.

*Helicobacter pylori* (*H. pylori*), a gram-negative bacterium, colonizes the stomach and causes chronic gastritis that mostly remains asymptomatic¹. *H. pylori* causes ulcers in the stomach or duodenum only in a subset of infected people², and is a risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma²,³,⁴. *H. pylori* is involved in extragastric disorders such as iron deficiency anemia⁵ and idiopathic thrombocytopenic purpura⁶. We and others have shown positive associations of *H. pylori* infection and its-related gastroduodenal morbidity with diabetes mellitus⁷–⁹, metabolic syndrome¹⁰,¹¹ and glycated hemoglobin (HbA1c) levels¹². Possible explanations for these relations include changes in gastric physiology induced by *H. pylori*. For example, *H. pylori* infection was shown to be related to ghrelin and leptin levels, two hormones that are expressed in the stomach and that play a major role in energy expenditure¹³–¹⁵. Chronic inflammation can contribute to the development of insulin resistance, which is pivotal in the pathophysiology of type 2 diabetes and metabolic syndrome¹⁶. Understanding the role of *H. pylori* infection in metabolic homeostasis and glycemic control in individuals with diabetes is paramount given the high burden of diabetes and its complications¹⁷ and the high *H. pylori* infection prevalence¹.

Studies that examined differences in HbA1c and fasting glucose levels among individuals with diabetes according to *H. pylori* infection usually had small sample sizes¹⁸–²², and some included both individuals with type 1 and type 2 diabetes mellitus. Various methods of detecting *H. pylori* were used, including serological assays²², the urea breath test (UBT) and invasive examinations based on gastric biopsies¹⁸,¹⁹. Evidence from these studies remains conflicting. One meta-analysis that included 14 studies (N = 1781 patients with diabetes) showed no significant difference in HbA1c levels between *H. pylori* infected and uninfected patients²³, while
another meta-analysis showed higher HbA1c levels among individuals who were *H. pylori* positive compared to negative; the weighted mean difference was 0.43, *P* = 0.02.

The aim of this study was to compare HbA1c, fasting plasma glucose and cholesterol levels between *H. pylori* infected and uninfected persons with diabetes, independent of potential confounders. Our hypothesis was that compared to those uninfected, those infected with *H. pylori* would have higher fasting blood glucose, total cholesterol and low-density lipoprotein (LDL) levels; and lower high-density lipoprotein (HDL). Additionally, we hypothesized a greater proportion of individuals with higher HbA1c (>7%) among those infected with *H. pylori*.

**Materials and methods**
**Study design and population.** A cross-sectional study was conducted using de-identified data from the computerized database of Maccabi Healthcare Services, the second largest health maintenance organization (HMO) in Israel. The study design was described elsewhere. Briefly, the study population comprised 147,936 persons who performed the UBT between 2002 and 2012. The age range at the UBT was 25–95 years; the mean was 42.8 years (standard deviation [SD] 12.7). Study exclusion criteria were determined based on factors that might affect the UBT result or metabolic control. Individuals were excluded if they purchased anti-*H. pylori* eradication therapy or proton pump inhibitors four weeks prior the UBT, had a cancer diagnosis within three years from the UBT or had documentation of bariatric surgery. Persons who purchased antibiotics for infections other than *H. pylori* were not excluded from the study. The current analysis was based on 12,207 (8.3%) patients with diabetes.

Diabetes was defined using the diabetes registry of Maccabi Healthcare Services. Persons with at least one of the following criteria were classified as having diabetes: 1) HbA1c ≥7.25%; 2) glucose ≥200 mg/dL; 3) diabetes diagnosis in the medical record (ICD-9 codes), and HbA1c ≥6.5% or glucose >125 mg/dL; 4) two purchases of diabetes medications in the previous two months, these can be purchased by prescription only. The above data are routinely validated with primary care physicians.

The main independent variable was *H. pylori* infection: positive or negative. *H. pylori* infection was defined based on the UBT result. The UBT was performed in fasting conditions. Individuals were asked to drink 75 mg of labeled urea with 13C, in 200 mL of orange juice. Breath samples were collected before ingestion of the labeled urea (baseline) and 30 min thereafter. Expired breath samples were analyzed by a mass spectrometer automated breath 13C analyzer. The ratio between 13C and 12C was measured at both time points in expired breath samples. The ratio of 13CO2 to 12CO2 in expired breath samples was determined and expressed as delta over baseline of 13CO2. *H. pylori* positivity was defined as 13CO2 delta over baseline > 3.5 per thousand. The sensitivity and specificity of UBT were estimated at 96% and 93%, respectively.

Covariates Potential confounders were selected based on evidence of their possible associations with *H. pylori* infection, glycemic control or cholesterol levels.

These included demographics: age (a continuous variable), sex, country of birth (classified as: Israel, the former Soviet Union, North Africa/Asia, Europe/Americas, and other/unknown) and residential socioeconomic status (SES). The presence of dyslipidemia was defined based on the International Classification of Disease codes-9th revision with clinical modifications (ICD-9-CM). Data were obtained on smoking (classified as ever, never, and unknown), body mass index (BMI) (weight in kilograms (kg)/height2 in meters (m)), and purchases of statins and diabetes medications (supplementary 1) one year prior to and one year after performing UBT. Each treatment was categorized as: 1) persistent use (three purchases with three-to-five-week interval between each two consecutive purchases); 2) non-persistent (purchases that did not meet the definition of persistent use), and 3) no purchases of these medications.

**Statistical analysis.** Descriptive statistics using frequencies and percentages, or means and standard errors, were employed to describe the study sample. Initially we examined differences between individuals who tested positive and negative for *H. pylori*, in sociodemographic and clinical factors, using the Student's *t* test for continuous variables, chi square tests for categorical variables, generalized linear models with binominal negative distribution and a log function link.

To account for potential confounders in the associations of *H. pylori* infection with glycemic control and cholesterol levels, we used the inverse probability of treatment weighting approach. We created a propensity score for the main independent variable using the predicted probability of *H. pylori* positivity from a logistic regression model with the abovementioned covariates. Inverse probability weights were calculated using the propensity score created by weighting each participant in the above-defined positivity categories (*H. pylori* positive or *H. pylori* negative), inversely to his/her probability of being classified into these specific categories. This created a pseudopopulation, which achieved a balance between the independent variable in the distribution of a given covariate. We compared the proportions of individuals who were *H. pylori* positive and negative for each of the covariates examined, using weighted generalized estimating equations (GEE) with binominal negative distribution and a log function link. This provided a robust variance estimator.

Next, in an unweighted analysis, we assessed differences in the median levels of glucose and HbA1c between individuals who were *H. pylori* positive and *H. pylori* negative, using the Mann–Whitney *U* test. We used the chi square test to assess differences between the groups in the proportion with worse glycemic control (HbA1c >7%). To account for confounders, as mentioned, the weighted GEE provides a robust variance estimator, which was used to compare between individuals who were *H. pylori* positive and negative, the proportion with worse...
glycemic control (HbA1c > 7%). We also conducted unweighted multivariable generalized linear models that adjusted for confounders using the conventional approach. Prevalence ratios and 95% confidence intervals (CIs) were obtained from these models. We followed a similar strategy in comparing cholesterol levels between individuals who were *H. pylori* positive and *H. pylori* negative. Here, we initially using the Student's *t* test to assess differences between the groups in mean levels. To account for confounders, we used weighted GEE with linear models. We also conducted unweighted analysis by means of multiple linear regression models that included *H. pylori* infection, the independent variable, as a dummy variable; and sociodemographic and clinical variables (age, country of birth, the use of diabetes medications and statins, smoking and BMI) were covariates. Beta coefficients (the slope) and the corresponding 95% CI and R² were obtained from the models. The assumptions of linear regression were met in the models of cholesterol levels. Differences between persons with and without *H. pylori* infection, in HbA1c, fasting glucose and cholesterol levels, were examined according to sociodemographic and clinical variables, using the Student's *t* test when comparing two groups, and one-way analysis of variance (ANOVA) when comparing three groups or more. The assumption of equal variance was examined using Levene's test; and when this was not met, we used the Welch's test. Correlations between age, and each of the dependent variables were examined using Pearson's correlation coefficient. Overall and sex-stratified analyses were performed. Interactions were examined of *H. pylori* infection with sex. Variables with missing data were handled using the missing indicator approach. Data were analysed using IBM-SPSS version 27 (IBM, Armonk, New York, USA). *P* < 0.05 was considered statistically significant. All statistical tests were 2-sided.

**Ethical consideration.** The study protocol was approved by the Helsinki committee of Assuta Medical Center and the ethics committee of Tel Aviv University. Since this is a retrospective study that used coded (anonymized) administrative data from electronic medical records, the requirement for informed consent was waived by the Helsinki committee.

We confirm that all methods were performed in accordance with the relevant guidelines and regulations.

**Results**

Of the 12,207 individuals with diabetes that comprised the cohort, 6,108 (50.0%) were positive for *H. pylori*. The mean age was significantly lower of those who were *H. pylori*-positive than *H. pylori*-negative: 54.4 years (SD = 11.7) vs. 57.9 (SD = 11.9). An inverse association was found between residential socioeconomic level and *H. pylori* infection. Individuals who were born in Europe and the Americas were less likely to have *H. pylori* infection than were those born in Israel. The proportion of individuals defined as persistent users of statins was lower among those who tested *H. pylori* positive than those who tested negative (50.4% vs. 59.9%). These differences were balanced in the weighted analysis (Table 1).

Differences in fasting blood glucose, HbA1c and cholesterol levels according to demographic and clinical factors appear in supplementary 2; Tables 1 and 2.

**Associations of *H. pylori* infection with fasting blood glucose and HbA1c levels.** Unweighted analysis showed no significant differences between individuals who were *H. pylori* positive and negative, in median HbA1c and glucose levels. A higher proportion of *H. pylori* infected men than uninfected men had worse glycemic control (HbA1c > 7%): 30.1% vs. 26.8%, *P* = 0.028. Such difference was not found in women (Table 2), heterogeneity chi square = 4.07, *P* = 0.044.

A weighted GEE model included worse control of diabetes (HbA1c > 7%) as the dependent variable. The independent variables were *H. pylori* infection, sex and the interaction term between sex and *H. pylori* infection. For men, but not women, this model showed a higher likelihood of worse glycemic control among individuals who tested positive versus negative for *H. pylori* prevalence ratio 1.11 (95% CI 1.00, 1.24), *P* = 0.043. A similar but non-statistically significant (*P* = 0.083) result was obtained in an unweighted multivariable model that adjusted for sociodemographic and clinical factors (Table 3).

**Associations of *H. pylori* infection with cholesterol levels.** Compared to persons uninfected, for persons who were infected with *H. pylori*, an unweighted analysis showed a higher mean total cholesterol level by 4.3 mg/dL (95% CI 2.5, 6.1), and a higher mean LDL level by 4.1 mg/dL (95% CI 2.6, 5.7). Similar findings were obtained in a stratified analysis by sex (Table 3).

In weighted GEE linear models, the differences between *H. pylori* infected and uninfected persons in total cholesterol and LDL cholesterol levels were attenuated and became non-statistically significant in men (Table 5). Adjustment for confounders using traditional linear regression models showed similar results to those obtained in the weighted models (Table 6). No significant difference was found between persons infected and uninfected with *H. pylori* in HDL level (Tables 4, 5, 6).

**Discussion**

We examined among persons with diabetes, differences in HbA1c, fasting plasma glucose and cholesterol levels, between those infected and not infected with *H. pylori*. To the best of our knowledge, this is the largest study to address this issue, with 12,207 individuals with diabetes.

The likelihood of having a high HbA1c (> 7.0%) was greater among *H. pylori* infected men than uninfected men. Such difference was not found in women (*P* for interaction 0.04). Mean total cholesterol and LDL cholesterol levels were higher among men infected with *H. pylori* than among individuals uninfected of both sexes. The results were consistent after adjustment for confounders using the inverse probability of treatment weighting approach and conventional multivariable models.
| Unweighted analysis | Weighted analysis |
|---------------------|-------------------|
| **H. pylori positive** | **H. pylori negative** | **H. pylori positive** | **H. pylori negative** |
| N = 6108 | N = 6099 | N = 6108 | N = 6099 |
| Mean age, years (SD) | 54.4 (11.7) | 57.9 (11.9) | 0.99 (0.98, 0.99) | < 0.001 | 56.1 (11.8) | 56.0 (12.1) | 1.00 (0.99, 1.00) | 0.88 |
| **Sex** | | | | | | | | |
| Male | 2926 (47.9%) | 2847 (46.7%) | 1 | 47.2% | 47.2% | 1 |
| Female | 3182 (52.1%) | 3252 (53.3%) | 0.97 (0.92, 1.04) | 0.43 | 52.8% | 52.8% | 1.00 (0.96, 1.04) | 0.99 |
| **Residential SES** | | | | | | | | |
| Low (1–5) | 3111 (51.0%) | 2583 (42.4%) | 1 | 46.5% | 46.4% | 1 |
| Intermediate (6–7) | 1541 (25.2%) | 1685 (27.6%) | 0.87 (0.81, 0.94) | 0.0043 | 26.6% | 26.6% | 1.00 (0.96, 1.02) | 0.99 |
| High (8–10) | 1167 (19.1%) | 1552 (25.4%) | 0.79 (0.72, 0.85) | 6.0E–09 | 22.2% | 22.3% | 0.99 (0.95, 1.05) | 0.95 |
| Missing | 289 (4.7%) | 279 (4.6%) | 0.93 (0.80, 1.08) | 0.34 | 4.7% | 4.7% | 1.00 (0.92, 1.09) | 0.98 |
| **Country of birth** | | | | | | | | |
| Israel | 3196 (52.3%) | 3080 (50.5%) | 1 | 51.4% | 51.5% | 1 |
| Former Soviet Union | 1838 (30.1%) | 1659 (27.2%) | 1.03 (0.96, 1.11) | 0.38 | 28.4% | 28.4% | 1.00 (0.96, 1.04) | 0.96 |
| North Africa/Asia | 524 (8.6%) | 480 (7.9%) | 1.02 (0.91, 1.15) | 0.067 | 8.3% | 8.3% | 1.00 (0.94, 1.07) | 0.96 |
| Europe/ Americas | 347 (5.7%) | 595 (9.8%) | 0.72 (0.63, 0.82) | 1.1E–06 | 7.8% | 7.8% | 1.00 (0.94, 1.08) | 0.87 |
| Other/unknown | 203 (3.3%) | 285 (4.7%) | 0.82 (0.69, 0.97) | 0.019 | 4.0% | 4.0% | 1.00 (0.92, 1.09) | 0.97 |
| Mean BMI, kg/m², SD | 30.4 (5.0) | 30.1 (5.0) | 1.01 (1.00, 1.01) | 0.056 | 30.2 (5.0) | 30.2 (5.1) | 1.00 (0.99, 1.01) | 0.99 |
| **Smoking** | | | | | | | | |
| Ever | 1010 (16.5%) | 859 (14.1%) | 1 | 15.3% | 15.3% | 1 |
| Never | 3591 (58.8%) | 3742 (61.3%) | 0.91 (0.83, 0.99) | 0.025 | 60.0% | 60.0% | 1.00 (0.95, 1.06) | 0.92 |
| Unknown | 1507 (24.7%) | 1498 (24.6%) | 0.93 (0.84, 1.02) | 0.13 | 24.7% | 24.7% | 0.99 (0.94, 1.06) | 0.95 |
| **Dyslipidemia, yes** | 4336 (71.0%) | 4613 (75.6%) | 0.89 (0.83, 0.95) | 0.001 | 73.6% | 73.5% | 1.12 (1.08, 1.17) | 0.93 |

| **Table 2.** Glucose and HbA1c levels of individuals with diabetes, according to H. pylori positivity, an unweighted analysis. IQR: interquartile range. *P value was obtained by the Mann–Whitney U test. bχ² test. cHeterogeneity χ² test = 4.074, P = 0.044 for the difference between men and women in the association between H. pylori infection and an HbA1c level > 7%. |
|---------------------|-----------------|
| HbA1c (%), median, IQR | 6.4 (1.1) | 6.4 (1.1) | 0.9* |
| HbA1c > 7.0%, N (%) | 1071 (26.9%) | 1025 (25.9%) | 0.2² |
| Glucose level (mg/dL), median, IQR | 118.0 (32.0) | 119.0 (31.0) | 0.2² |

| **Table 1.** Sociodemographic and clinical characteristics of individuals with diabetes, according to H. pylori positivity – unweighted and weighted analyses. BMI: body mass index, CI: confidence interval, kg: kilogram, m: meters, PR: prevalence ratio, SD: standard deviation, SES: socioeconomic status. a PR, 95% CI and P values were obtained using a univariable general linear model with negative binomial distribution; P value for the PR. bPR, 95% CI and P values were obtained using weighted univariable generalized estimating equation models with negative binomial distribution; P value for the PR. cBased on purchasing medications. |
showed no significant difference in total cholesterol, LDL and HDL levels among individuals with diabetes. 

A study from Germany found higher HDL cholesterol levels in infected persons than uninfected ones. This result is in agreement with findings reported among infected persons with diabetes mostly reported no significant differences. However, direct comparability between our and other studies might be limited due to methodological differences. Usually, men had a higher risk than women for worse control of diabetes (HbA1c > 7%), and the independent variables, H. pylori infection, sex and the interaction term sex were H. pylori infection, sex and the interaction term sex (1 = women) by H. pylori infection. Model 2 is adjusted also for the variables age, body mass index (both continuous variables), residential socioeconomic rank, diagnosis of dyslipidemia, and the use of statins and diabetes medications. A generalized estimating equation model with negative binomial distribution and log function link. The dependent variable was worse control of diabetes (HbA1c > 7%), and the independent variables, H. pylori infection, sex and the interaction term sex (1 = women) by H. pylori infection. No significant interaction was found between age and H. pylori infection, P = 0.17.

### Table 3.
The association of H. pylori infection with worse control of diabetes (HbA1c > 7%). CI: confidence intervals; PR: prevalence ratio. General linear model with negative binomial distribution and a log function link. The dependent variable was worse control of diabetes (HbA1c > 7%). The independent variables were H. pylori infection, sex and the interaction term sex (1 = women) by H. pylori infection. Model 2 is adjusted also for the variables age, body mass index (both continuous variables), residential socioeconomic rank, diagnosis of dyslipidemia, and the use of statins and diabetes medications. A generalized estimating equation model with negative binomial distribution and log function link. The dependent variable was worse control of diabetes (HbA1c > 7%), and the independent variables, H. pylori infection, sex and the interaction term sex (1 = women) by H. pylori infection. No significant interaction was found between age and H. pylori infection, P = 0.17.

### Table 4.
Mean cholesterol levels of individuals with diabetes, according to H. pylori infection, an-unweighted analysis. CI: confidence interval, HDL: high-density lipoproteins, LDL: low-density lipoproteins, SE: standard error. The mean difference between H. pylori infected and uninfected persons with diabetes. P value was obtained by Student's t test. The assumption of equal variance between H. pylori positive and negative individuals was met, as determined by Levene's F test, for all tested parameters.

|                  | H. pylori positive, Number | Mean (SE) | H. pylori negative, Number | Mean (SE) | Mean differencea (95% CI) | PR (95% CI) | P valueb |
|------------------|---------------------------|-----------|---------------------------|-----------|--------------------------|-------------|---------|
| Overall          | 6104                      | 6103      |                           |           |                          |             |         |
| Total cholesterol (mg/dL) | 4663                  | 199.0 (0.65) | 4653                     | 194.7 (0.64) | 4.3 (2.5, 6.1) | 2.5E−06     |         |
| LDL (mg/dL)     | 4605                      | 120.0 (0.55) | 4612                     | 115.9 (0.55) | 4.1 (2.6, 5.7) | 1.3E−07     |         |
| HDL (mg/dL)     | 4650                      | 46.9 (0.17)   | 4637                     | 47.4 (0.18)   | 0.5 (0.2, 1.0) | 0.04        |         |
| Men              | 2923                      | 2850      |                           |           |                          |             |         |
| Total cholesterol (mg/dL) | 2184                  | 193.3 (0.98) | 2129                     | 186.9 (0.93) | 6.4 (3.8, 9.0) | 2.0E−06     |         |
| LDL (mg/dL)     | 2144                      | 117.7 (0.81) | 2103                     | 112.4 (0.81) | 5.3 (3.0, 7.5) | 5.0E−06     |         |
| HDL (mg/dL)     | 2178                      | 42.3 (0.21)   | 2119                     | 42.6 (0.21)   | − 0.3 (− 0.9, 0.3) | 0.3         |         |
| Women            | 3181                      | 3253      |                           |           |                          |             |         |
| Total cholesterol (mg/dL) | 2475                  | 204.0 (0.86) | 2528                     | 201.3 (0.86) | 2.7 (0.3, 5.1) | 0.02        |         |
| LDL (mg/dL)     | 2457                      | 122.1 (0.75) | 2513                     | 118.8 (0.74) | 3.3 (1.2, 5.3) | 0.002       |         |
| HDL (mg/dL)     | 2468                      | 51.0 (0.24)   | 2522                     | 51.5 (0.24)   | − 0.5 (− 0.2, 1.2) | 0.1         |         |

Previous studies that assessed differences in HbA1c and fasting glucose levels between H. pylori infected and uninfected persons with diabetes mostly reported no significant differences. However, direct comparability between our and other studies might be limited due to methodological differences. Usually, men had a higher risk than women for H. pylori-related gastro-duodenal diseases such as peptic disease and gastric cancer. Therefore, the association of H. pylori with HbA1c levels in men only might not be surprising. Interestingly, Chen and Blaser reported positive associations between H. pylori sero-prevalence and HbA1c in a well-designed study of a large US general population sample; this supports our finding.

The observed difference in HbA1c levels by H. pylori infection in men is of modest magnitude. HbA1c is a measure of diabetes control and is associated with diabetic cardiovascular complications and mortality. Therefore, identifying modifiable factors that can affect HbA1c level is highly valuable. If H. pylori is causally related to HbA1c levels, then anti-H. pylori therapy is expected to affect HbA1c. Randomized controlled trials are needed to test this hypothesis. Even if the observed association between H. pylori and HbA1c in men with diabetes is not causal, our findings are still clinically important and can be useful in identifying persons at risk for less favorable glycemic control.

Interestingly, in both men and women, significantly higher total cholesterol and LDL cholesterol levels were found among H. pylori infected persons than uninfected ones. This result is in agreement with findings reported by Laurila et al. Vafaieimansh et al. showed higher HDL cholesterol levels in H. pylori infected vs. uninfected persons with diabetes, but no significant differences in total cholesterol and LDL levels. A study from Germany showed no significant difference in total cholesterol, LDL and HDL levels among individuals with diabetes.
according to *H. pylori* sero-prevalence. However, worse lipid profile was found in healthy persons infected with *H. pylori* than uninfected ones, when UBT was used to determine the presence of the infection. The association between cholesterol levels and cardiovascular disease is well established, and maintaining low LDL cholesterol is recognized as important for the prevention of cardiovascular disease. Since diabetes is a risk factor for cardiovascular disease, the importance of maintaining optimal cholesterol levels is amplified in this context. Hence, our findings might serve as a basis for future clinical trials that assess whether *H. pylori* eradication can affect blood cholesterol levels in individuals with diabetes. Moreover, *H. pylori* infection might be a useful marker to identify persons with diabetes at risk for worse blood lipid levels.

The mechanism by which *H. pylori* infection might affect HbA1c and blood cholesterol levels is not fully clear, but might be related to the inflammation induced by the infection. *H. pylori* colonization in the stomach triggers a humoral and cellular immune response. The response of the cellular immune system is induced through T-helper-1 cells that release pro-inflammatory cytokines and activation of phagocytosis. *H. pylori* also activates T-helper 2 response and regulatory T-cells. Inflammatory agents such as cytokines and increased production of reactive oxygen species inhibit insulin signaling.

### Table 5. Cholesterol levels by sex -weighted generalized estimating equation models in individuals with diabetes, according to *H. pylori* infection. CI: confidence interval, HDL: high-density lipoproteins, LDL: low-density lipoproteins, SE: standard error. The weights were determined based on the inverse probability of treatment weighting approach. The weighted analysis was performed using generalized estimating equations with linear models. Beta coefficients, 95% CIs, P values, mean levels and standard errors in individuals with diabetes, according to *H. pylori* infection were obtained from these models.

|                | *H. pylori* positive, Mean (SE) | *H. pylori* negative, Mean (SE) | Beta coefficient (95% CI) | P value |
|----------------|-------------------------------|-------------------------------|--------------------------|---------|
| **Overall**    |                               |                               |                          |         |
| Total cholesterol (mg/dL) | 198.2 (0.67) | 195.5 (0.65) | 2.7 (0.8, 4.5) | 0.004   |
| LDL (mg/dL)    | 119.1 (0.59) | 116.9 (0.56) | 2.2 (0.6, 3.8) | 0.006   |
| HDL (mg/dL)    | 47.3 (0.18)  | 47.1 (0.18)  | 0.2 (−0.3, 0.7) | 0.3     |
| **Men**        |                               |                               |                          |         |
| Total cholesterol (mg/dL) | 191.5 (0.98) | 188.9 (0.98) | 2.6 (−0.06, 5.4) | 0.055   |
| LDL (mg/dL)    | 116.2 (0.83) | 114.2 (0.85) | 2.0 (−0.2, 4.3) | 0.087   |
| HDL (mg/dL)    | 42.5 (0.22)  | 42.3 (0.21)  | 0.2 (−0.4, 0.8) | 0.4     |
| **Women**      |                               |                               |                          |         |
| Total cholesterol (mg/dL) | 203.9 (0.85) | 201.2 (0.85) | 2.8 (0.4, 5.2) | 0.025   |
| LDL (mg/dL)    | 121.6 (0.78) | 119.2 (0.75) | 2.4 (0.3, 4.5) | 0.028   |
| HDL (mg/dL)    | 51.4 (0.25)  | 51.1 (0.25)  | 0.3 (−0.4, 0.9) | 0.3     |

### Table 6. Multivariable linear regression models of associations of *H. pylori* infection with cholesterol levels in individuals with diabetes, an unweighted analysis. Each model was adjusted for age, body mass index (both continuous variables), sex, residential socioeconomic rank, diagnosis of dyslipidemia, and the use of statins and diabetes medications (dummy variables). Adjusted $R^2$ = 0.14; Adjusted $R^2$ = 0.07; Adjusted $R^2$ = 0.12. In all models, the values of variance inflation factor (VIF) ranged from 1.03 to 2.05, suggesting no multicollinearity. Significant interactions were not found between age and *H. pylori* infection, and between sex and *H. pylori* infection.

| *H. pylori* infection (positive vs. negative) | Unstandardized beta coefficient (95% CI) | P value |
|---------------------------------------------|----------------------------------------|---------|
| **Overall**                                 |                                        |         |
| Total cholesterol (mg/dL)                   | 3.0 (1.3, 4.7)                         | 0.001   |
| LDL cholesterol (mg/dL)                     | 2.5 (1.1, 3.9)                         | 0.001   |
| HDL cholesterol (mg/dL)                     | 0.3 (−0.1, 0.8)                        | 0.1     |
| **Men**                                     |                                        |         |
| Total cholesterol (mg/dL)                   | 3.5 (0.9, 6.0)                         | 0.007   |
| LDL cholesterol (mg/dL)                     | 2.8 (0.6, 4.9)                         | 0.011   |
| HDL cholesterol (mg/dL)                     | 0.3 (−0.3, 0.9)                        | 0.2     |
| **Women**                                   |                                        |         |
| Total cholesterol (mg/dL)                   | 2.4 (0.2, 4.7)                         | 0.035   |
| LDL cholesterol (mg/dL)                     | 2.1 (0.1, 4.1)                         | 0.036   |
| HDL cholesterol (mg/dL)                     | 0.3 (−0.4, 0.9)                        | 0.3     |
Our study has some limitations. We used data from a large HMO database, which were collected for clinical care. The methods of collecting information on variables such as BMI and smoking may vary among physicians and nurses. Information was missing for some variables. For example, smoking status was unknown for 25% of the cohort. Data were collected of individuals who were referred to the UBT by their physicians. This implies that only those who had gastrointestinal symptoms were included.

Our study also has several strengths. The study was conducted using a large database of the second largest HMO in Israel, which insures about 25% of Israel's population; this is expected to increase the generalizability of the findings. The status of \( H. \text{pylori} \) infection was determined by UBT, which was shown to have high sensitivity and specificity, 96% and 93%, respectively\(^{28}\). In addition, levels of HbA1c, fasting blood glucose and cholesterol levels were tested in one laboratory. Lastly, the presence of diabetes was determined based on a combination of physician's diagnosis, laboratory results and purchasing of diabetes medications; this mitigates misclassification bias.

In conclusion, for men but not women, the likelihood of worse control of HbA1c was higher in persons who tested positive than negative for \( H. \text{pylori} \). Higher total cholesterol and LDL cholesterol levels were found among men with \( H. \text{pylori} \) infection than among individuals of both sexes without infection. These associations were maintained after adjustment for potential confounders using a number of analytical methods. Our findings might have implications on glycemic control and the management of cholesterol levels in individuals with diabetes, and on the prevention of diabetes complications.

Data availability
The datasets generated during and/or analyzed during the current study are not publicly available due to local legal restriction and regulations.

Received: 24 October 2019; Accepted: 30 March 2021
Published online: 19 April 2021

References
1. Suerbaum, S. & Michetti, P. Helicobacter pylori infection. N. Engl. J. Med. 347, 1175–1186. https://doi.org/10.1056/NEJMra020542 (2002).
2. Nomura, A. M. Y., Perez-Perez, G. I., Lee, J., Stemmermann, G. & Blaser, M. J. Relation between Helicobacter pylori infection and gastric-carcinoma among Japanese-Americans in Hawaii. N. Engl. J. Med. 325, 1132–1136. https://doi.org/10.1056/NEJM199101173251604 (1991).
3. Parsonnet, J. et al. Helicobacter pylori infection and gastric lymphoma. N. Engl. J. Med. 330, 1267–1271. https://doi.org/10.1056/NEJM19940503301803 (1994).
4. Hudak, L., Jaraisy, A., Haj, S. & Muhsen, K. An updated systematic review and meta-analysis on the association between Helicobacter pylori infection and iron deficiency anemia. Helicobacter https://doi.org/10.1111/hel.12330 (2017).
5. Kuwana, M. Effect of Helicobacter pylori infection and its related gastroduodenal morbidity with metabolic syndrome: a systematic review. J. Gastroenterol. Hepatol. 34, 205–211. https://doi.org/10.1111/j.1440-1758.2008.05472.x (2009).
6. Kuwana, M. Helicobacter pylori-associated immune thrombocytopenia: clinical features and pathogenic mechanisms. World J. Gastroenterol. 20, 714–725. https://doi.org/10.3748/wjg.v20.i3.714 (2014).
7. Haj, S. et al. Associations of Helicobacter pylori infection and peptic disease with diabetic mellitus: results from a large population-based study. PLoS ONE 12, e0181687. https://doi.org/10.1371/journal.pone.0181687 (2017).
8. Bener, A. et al. Association between type 2 diabetes mellitus and Helicobacter pylori infection. Turk. J. Gastroenterol. 18, 225–229 (2007).
9. Zhou, X., Zhang, C., Wu, J. & Zhang, G. Association between Helicobacter pylori infection and diabetes mellitus: a meta-analysis of observational studies. Diab. Res. Clin. Pract. 99, 200–208. https://doi.org/10.1016/j.diabres.2012.11.012 (2013).
10. Refaeli, R. et al. Relationships of \( H. \text{pylori} \) infection and its related gastroduodenal morbidity with metabolic syndrome: a large cross-sectional study. Sci. Rep. 8, 4088. https://doi.org/10.1038/s41598-018-22198-9 (2018).
11. Upala, S., Jaruvongvanich, T., Rungwitat, T., Jaruvongvanich, S. & Sanguansen, A. Association between Helicobacter pylori infection and metabolic syndrome: a systematic review and meta-analysis. J. Dig. Dis. 17, 433–440. https://doi.org/10.1111/1751-2980.12367 (2016).
12. Chen, Y. & Blaser, M. J. Association between gastric Helicobacter pylori colonization and glycedated hemoglobin levels. J. Infect. Dis. 205, 1195–1200. https://doi.org/10.1093/infdis/jis106 (2012).
13. Francios, F. et al. The effect of \( H. \text{pylori} \) eradication on meal-associated changes in plasma ghrelin and leptin. BMC Gastroenterol. 11, 37. https://doi.org/10.1186/1471-230X-11-37 (2011).
14. Breidert, M. et al. Leptin and its receptor in normal human gastric mucosa and in Helicobacter pylori-associated gastritis. Scand. J. Gastroenterol. 34, 954–961. https://doi.org/10.1080/00365529970025039 (1999).
15. Chuang, C. H. et al. Gender difference of circulating ghrelin and leptin concentrations in chronic Helicobacter pylori infection. Helicobacter 14, 54–60. https://doi.org/10.1111/j.1533-2907.2009.00653.x (2009).
16. Wellen, K. E. & Hotamisligil, G. S. Inflammation, stress, and diabetes. J. Clin. Invest. 115, 1111–1119. https://doi.org/10.1172/JCI25102 (2005).
17. van Dieren, S., Beulens, J. W., van der Schouw, Y. T., Grobbee, D. E. & Neal, B. The global burden of diabetes and its complications: an emerging pandemic. Eur. J. Cardiovasc. Prev. Rehabil. 17(Suppl 1), S3–8. https://doi.org/10.1097/01.hjr.0000368191.86614.5a (2010).
18. Ko, G. T. et al. Helicobacter pylori infection in Chinese subjects with type 2 diabetes. Endocr. Res. 27, 171–177. https://doi.org/10.1081/erc-100107178 (2001).
19. Demir, M. et al. Helicobacter pylori prevalence in diabetes mellitus patients with dyspeptic symptoms and its relationship to glycemic control and late complications. Dig. Dis. Sci. 53, 2646–2649. https://doi.org/10.1007/s10620-007-0185-7 (2008).
20. Jones, K. L. et al. Helicobacter pylori infection is not associated with delayed gastric emptying or upper gastrointestinal symptoms in diabetes mellitus. Dig. Dis. Sci. 47, 704–709. https://doi.org/10.1023/A:1014763210890 (2002).
21. Vafeiamanesh, J., Bagherzadeh, M., Heidari, A., Motfi, F. & Parham, M. Diabetic patients infected with Helicobacter pylori have a higher insulin resistance degree. Cyspian J. Internal Med. 5, 137–142 (2014).
22. Haj, S., Raviv, M. & Muhsen, K. in Extradigestive Manifestations of Helicobacter pylori Infection—An Overview (ed Roesler B.M.) 141–161 (INTECH, 2016).
23. Horikawa, C. et al. Association of Helicobacter pylori infection with glycemic control in patients with diabetes: a meta-analysis. J. Diabetes Res. 2014, 250620. https://doi.org/10.1155/2014/250620 (2014).
24. Dai, Y. N., et al. Is Helicobacter pylori infection associated with glycemic control in diabetics? World J Gastroenterol. 21, 5407–5416. https://doi.org/10.3748/wjg.v21.i17.5407 (2015).
25. Heymann, A. D. et al. The implementation of managed care for diabetes using medical informatics in a large Preferred Provider Organization. Diabetes Res. Clin. Pract. 71, 290–298. https://doi.org/10.1016/j.diabres.2005.07.002 (2006).
26. Heymann, A. D., Chodick, G., Halkin, H., Kokia, E. & Shalev, V. Description of a diabetes disease register extracted from a central database. Rarefactual 146, 15–17 (2007).
27. Shмуэл, H., Yahav, J., Samra, Z., Chodick, G. & Ofek, I. Elevated 13C urea breath test values females infected with Helicobacter pylori. Dig. Dis. Sci. 52, 402–404. https://doi.org/10.1007/s10620-006-9590-6 (2007).
28. Gisbert, J. P. & Pajares, J. M. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection—a critical review. Aliment. Pharmacol. Ther. 20, 1001–1017. https://doi.org/10.1111/j.1365-2036.2004.02203.x (2004).
29. Israel Central Bureau of Statistics. State of Israel (Israel Central Bureau of Statistics, Jerusalem, 2013).
30. Austin, P. C. & Stuart, E. A. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. Stat. Med. 34, 3661–3679. https://doi.org/10.1002/sim.6607 (2015).
31. Rosenbaum, P. R. & Rubin, D. B. The central role of the propensity score in observational studies for causal effects. Biometrika 70, 41–55. https://doi.org/10.1093/biomet/70.1.41 (1983).
32. Desai, R. J. & Franklin, J. M. Alternative approaches for confounding adjustment in observational studies using weighting based on the propensity score: a primer for practitioners. BMJ 357, i5657. https://doi.org/10.1136/bmj.i5657 (2019).
33. Spreeuwenberg, M. D. et al. The multiple propensity score as control for bias in the comparison of more than two treatment arms: an introduction from a case study in mental health. Med. Care 48, 166–174. https://doi.org/10.1097/MC.0b013e3181c1328f (2010).
34. Austin, P. C. Variance estimation when using inverse probability of treatment weighting (IPTW) with survival analysis. Stat. Med. 35, 5642–5655. https://doi.org/10.1002/sim.7084 (2016).
35. Torre, L. A. et al. Global Cancer Statistics, 2012. CA Cancer J. Clin. 65, 87–108. https://doi.org/10.3322/caac.21262 (2015).
36. Schubert, T. T. et al. Ulcer risk factors—interactions between helicobacter-pylori infection, nonsteroidal use, and age. Am. J. Med. 94, 413–418. https://doi.org/10.1016/0002-9343(93)90153-G (1995).
37. Arnold, L. W. & Wang, Z. The HbA1c and all-cause mortality relationship in patients with type 2 diabetes is J-shaped: a meta-analysis of observational studies. Rev. Diabet. Stud. 11, 138–152. https://doi.org/10.1900/RDS.2014.11.138 (2014).
38. Alatorre, C. I. et al. Factors associated with stroke, myocardial infarction, ischemic heart disease, unstable angina, or mortality in patients from real world clinical practice with newly-diagnosed type 2 diabetes and early glycemic control. Curr. Med. Res. Opin. 34, 337–343. https://doi.org/10.1080/03007995.2017.1396969 (2018).
39. Laurila, A. et al. Association of Helicobacter pylori infection with elevated serum lipids. Atherosclerosis 142, 207–210. https://doi.org/10.1016/j.atherosclerosis.2004.09.004 (2004).
40. Ongey, M., Brenner, H., Thefeld, W. & Rothenbacher, D. Helicobacter pylori and hepatitis A virus infections and the cardiovascular risk profile in patients with diabetes mellitus: results of a population-based study. Eur. J. Cardiovasc. Prev. Rehabil. 11, 471–476. https://doi.org/10.1097/HJR.0b013e410147f730 (2004).
41. Hoffmeister, A. et al. Current infection with Helicobacter pylori, but not seropositivity to Chlamydia pneumoniae or cytomegalovirus, is associated with an atherogenic, modified lipid profile. Arterioscl. Throm. Vas. 21, 427–432. https://doi.org/10.1161/01.ATV.21.3.427 (2001).
42. Stone, N. J. et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J. Am. Coll. Cardiol. 63, 2889–2934. https://doi.org/10.1016/j.jacc.2013.11.002 (2014).
43. Altherton, J. C. The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. Ann. Rev. Pathol. 1, 63–96. https://doi.org/10.1146/annurev.pathol.1.110304.100125 (2006).
44. Altherton, J. C. & Blaser, M. J. Coadaptation of Helicobacter pylori and humans: ancient history, modern implications. J. Clin. Investig. 119, 2475–2487. https://doi.org/10.1172/JCI38605 (2009).

Acknowledgment
We thank Ms. Racheli Katz from Maccabi Health services for her help in retrieving the data from the computerized databases. This work was performed in partial fulfillment of the requirements for an MPH degree of Dr. Saeda Haj, the Sackler Faculty of Medicine, Tel Aviv University.

Author contributions
KM, GC and VS designed the study; KM was responsible for all aspects of the study; SH and KM were involved in data collection and management; SH, SG and KM analysed the data. All the authors contributed to the interpretation of the results; SH, WN and KM prepared the first draft of the manuscript. All the authors contributed to writing and revising the manuscript and approved the final version.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-87808-5.
Correspondence and requests for materials should be addressed to K.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
