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RESEARCH PAPER

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Prospective clinical trial of hepatitis B vaccination in adults with and without type-2 diabetes mellitus

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ABSTRACT

Objective: Patients with diabetes mellitus are at increased risk for hepatitis B virus (HBV) infection and its complications. HBV vaccination is recommended for adults with diabetes in the United States and other countries. However, few studies have assessed safety and immunogenicity of hepatitis B vaccine in such patients. We assessed the safety and immunogenicity of recombinant hepatitis B vaccine in subjects with and without diabetes mellitus.

Methods: Prospective, multi-country controlled study in 21 centers (www.clinicaltrials.gov NCT01627340). Four hundred and sixteen participants with Type-2 diabetes and 258 controls matched for age and body mass index (BMI) (2:1 ratio) received 3-doses of HBV vaccine (*Engerix*-B^{TM}, GSK Vaccines, Belgium) according to a 0, 1, 6 months schedule. Antibodies were measured against HBV surface antigen and expressed as seroprotection rates (anti-HBs $\geq$10mIU/mL) and geometric mean concentration (GMC).

Results: The median age and BMI in patients with diabetes and controls (according-to-protocol cohort) were 54 y and 32.1 kg/m^2, and 53 y and 30.8 kg/m^2, respectively. Seroprotection rates (GMCs) one month post-dose-3 were 75.4% (147.6 mIU/mL) and 82.0% (384.2 mIU/mL) in patients with diabetes and controls, respectively. Age-stratified seroprotection rates for patients with diabetes were 88.5% (20–39 years), 81.2% (40–49 years), 83.2% (50–59 years), and 58.2% ($\geq$60 years). The overall safety profile of hepatitis B vaccine was similar between groups.

Conclusions: Hepatitis B vaccine is immunogenic in patients with diabetes and has a similar safety profile to vaccination in healthy controls. Because increasing age was generally associated with a reduction in seroprotection rates, hepatitis B vaccine should be administered as soon as possible after the diagnosis of diabetes.

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Introduction

In the United States (US), patients with diabetes mellitus (DM) have twice the risk for developing acute hepatitis B virus (HBV) infections as healthy adults.[1] Furthermore, the seroprevalence of antibodies to HBV core antigen (anti-HBc) is 60% higher among patients with DM than those without DM.[2] Numerous HBV outbreaks among patients with DM have been recorded in the US and countries in Europe.[3-8] Of 29 outbreaks recorded since 1996 in US long-term care or assisted living facilities, 25 involved assisted blood glucose monitoring.[2]

In 2011 the US Advisory Committee on Immunization Practices (ACIP) recommended hepatitis B vaccine for adults with DM from 19 through 59 y of age, and for diabetic adults aged $\geq$60 y at the discretion of the treating clinician.[2] Similar recommendations have been made by professional medical associations in other countries, including Canada and the Czech Republic, and have been adopted by the Superior Council of Health in Belgium.[9-11]

Evidence suggests that seroprotection rates after hepatitis B vaccination are lower among adults with DM (reviewed in[12]), or who have renal disease.[13,14]

We therefore conducted a prospective, open, controlled study to determine the safety and immunogenicity of hepatitis B vaccination in patients with type-2 DM and to assess the impact of the confounding effects of age and obesity on the immune response.

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Supplemental data for this article can be accessed on the publisher's website to publisher's website.

Results

Demography

Eight-hundred and ninety-three individuals were screened and 674 were vaccinated (Fig. S1). The most common reason for screening failure was seropositivity for hepatitis B surface antigen (HBsAg), hepatitis B surface (anti-HBs) and/or to hepatitis B core (anti-HBc) antibodies (n = 104). Sixteen participants withdrew during the study (Fig. S1). There was one withdrawal due to a serious adverse event (SAE): a 45-year old man with DM was diagnosed with a malignant brain neoplasm after dose 2. The event was considered unrelated to vaccination.

One participant with diabetes was reported to be pregnant 5 months post-dose 2. This subject was withdrawn from the study and later gave birth to a live infant with no apparent congenital anomaly.

The median time since the diagnosis of type-2 DM was 935 d (range 7–1890 d). In the according-to-protocol (ATP) cohort, the median age of participants was 54.0 y in the diabetes group and 53.0 y in controls; 55.6% and 41.3% of participants in the respective groups were male (Table 1). There were 57.4% of participants with diabetes and 57.7% of control participants with a BMI ≥30.

Immunogenicity

One month after the 3 vaccine doses, the seroprotection (defined as anti-HBs ≥10mIU/mL) rate was 75.4% in the diabetes group and 82.0% in the control group (Table 2). In an exploratory analysis, the difference between groups in the seroprotection rate was 6.61% (95% CI [−0.70; 13.34]). The geometric mean concentration (GMC) ratio (diabetes group/control group) adjusted for country, age and body mass index (BMI) strata as fixed effects was 0.40 (95% CI [0.25; 0.64]).

Seroprotection rates decreased with age in diabetics as well as in controls (Table 2). In the diabetes group, the point estimate of the seroprotection rate was lower than controls for most age groups. In subjects with diabetes, the seroprotection rate was 79.5% (95% CI [72.4; 85.5]) for those with BMI <30 kg/m^2, versus 72.4% (95% CI [65.9; 78.2]) when the BMI was ≥30 kg/m^2 (Table 2). In controls, the seroprotection rate was similar (82.5% [95% CI 72.4; 90.1] vs. 81.7% [95% CI 73.1; 88.4]) in both BMI categories.

In the logistic regression modeling, age and BMI had a statistically significant negative effect on the likelihood of achieving seroprotection ($p < 0.0001$, Table 3) but not gender or whether the subject was diabetic or not. In the stepwise regression analysis, reduced anti-HBs antibody GMCs were associated with increased BMI, increased age, male gender and the presence of diabetes (Table 3).

Results of the total vaccinated cohort (TVC) analysis, performed on an unmatched population, were similar to those of the ATP cohort (data not shown). However the 95% CI for the group difference in seroprotection rates did not include 0 in that analysis, indicating that a difference between groups in the TVC cannot be ruled out.

Reactogenicity and safety

The most frequently reported solicited local symptom in each group was pain, and the most frequently reported

Table 1. Demographic characteristics at screening (according-to-protocol immunogenicity cohort).

Characteristic	Categories	Diabetes group N = 378	Control group N = 189
Age (years)	Median (range)	54.0 (20–82)	53.0 (21–81)
	Mean (SD)	53.7 (12.07)	53.1 (12.88)
Gender n(%)	Female	168 (44.4)	111 (58.7)
	Male	210 (55.6)	78 (41.3)
Geographic Ancestry n(%)	White - Caucasian / European Heritage	319 (84.4)	177 (93.7)
	African Heritage / African American	16 (4.2)	2 (1.1)
	American Indian or Alaskan Native	17 (4.5)	1 (0.5)
	Other*	26 (6.7)	9 (4.8)
BMI (kg/m^2)	Median (range)	32.1 (19.3–63.5)	30.8 (16.7–51.6)
	<30 n (%)	161 (42.6)	80 (42.3)
	≥30 n (%)	217 (57.4)	109 (57.7)
HbA1c (%)	Median (range)	6.5 (4.8–13.9)	5.5 (4.2–6.3)
HbA1c (mmol/mol)	Median (range)	48 (29–127)	37 (22–45)
eGFR (mL/min/1.73m^2)	Median (range)	88 (50–127)	82 (50–135)
	50–80 n (%)	131 (34.7)	86 (45.5)
	80–120 n (%)	239 (63.2)	100 (52.9)
	>120 n (%)	8 (2.1)	3 (1.6)
Age/BMI distribution n(%)	Age 20–39 y and <BMI 30 kg/m^2	14 (3.7)	7 (3.7)
	Age 20–39 y and ≥BMI 30 kg/m^2	38 (10.1)	19 (10.1)
	Age 40–49 y and <BMI 30 kg/m^2	26 (6.9)	13 (6.9)
	Age 40–49 y and ≥BMI 30 kg/m^2	59 (15.6)	31 (16.4)
	Age 50–59 y and <BMI 30 kg/m^2	61 (16.1)	31 (16.4)
	Age 50–59 y and ≥ BMI 30 kg/m^2	58 (15.3)	31 (16.4)
	Age ≥60 years and < BMI 30 kg/m^2	60 (15.9)	29 (15.3)
	Age ≥60 years and ≥ BMI 30 kg/m^2	62 (16.4)	28 (14.8)

N = total number of subjects.
n/% = number / percentage of subjects in a given category.
SD = standard deviation.
BMI = body mass index, HbA1c = glycated hemaglobin.
eGFR =Estimated Glomerular Filtration Rate.
*Other includes Asian, Japanese, Native Hawaiian or Other Pacific Islander, Arabic and North African Heritage.

Table 2. Antibody response to hepatitis B vaccination in the diabetes and control groups and in subgroups according to age and BMI, one month post-dose 3 (according-to-protocol immunogenicity cohort).

	Diabetes N = 378			Controls		
	N	n	% (95% CI)	N	n	% (95% CI)
All participants						
≥ 6.2 mIU/mL	378	293	77.5 (73.0; 81.6)	189	160	84.7 (78.7; 89.5)
≥ 10 mIU/mL	378	285	75.4 (70.7; 79.7)	189	155	82.0 (75.8; 87.2)
≥ 100 mIU/mL	378	203	53.7 (48.5; 58.8)	189	135	71.4 (64.4; 77.8)
GMC	378	—	147.6 (110.2; 197.8)	189	—	384.2 (254.5; 580.0)
Anti-HBs antibodies ≥ 10 mIU/mL by age						
Age 20–39 years	52	46	88.5 (76.6; 95.6)	26	26	100 (86.8; 100)
Age 40–49 years	85	69	81.2 (71.2; 88.8)	44	38	86.4 (72.6; 94.8)
Age 50–59 years	119	99	83.2 (75.2; 89.4)	62	51	82.3 (70.5; 90.8)
Age ≥60 years	122	71	58.2 (48.9; 67.1)	57	40	70.2 (56.6; 81.6)
Anti-HBs antibodies ≥ 10 mIU/mL by BMI						
BMI <30 kg/m^2	161	128	79.5 (72.4; 85.5)	80	66	82.5 (72.4; 90.1
BMI ≥30 kg/m^2	217	157	72.4 (65.9; 78.2)	109	89	81.7 (73.1; 88.4)
Anti-HBs antibodies ≥ 10 mIU/mL by age and BMI						
Age 20–39 years, BMI <30 kg/m^2	14	12	85.7 (57.2; 98.2)	7	7	100 (59.0; 100)
Age 20–39 years, BMI ≥30 kg/m^2	38	34	89.5 (75.2; 97.1)	19	19	100 (82.4; 100)
Age 40–49 years, BMI <30 kg/m^2	26	21	80.8 (60.6; 93.4)	13	13	100 (75.3; 100)
Age 40–49 years, BMI ≥30 kg/m^2	59	48	81.4 (69.1; 90.3)	31	25	80.6 (62.5; 92.5)
Age 50–59 years, BMI <30 kg/m^2	61	55	90.2 (79.8; 96.3)	31	26	83.9 (66.3; 94.5)
Age 50–59 years, BMI ≥30 kg/m^2	58	44	75.9 (62.8; 86.1)	31	25	80.6 (62.5; 92.5)
Age ≥60 years, BMI <30 kg/m^2	60	40	66.7 (53.3; 78.3)	29	20	69.0 (49.2; 84.7)
Age ≥60 years, BMI ≥30 kg/m^2	62	31	50.0 (37.0; 63.0)	28	20	71.4 (51.3; 86.8)

GMC = geometric mean antibody concentration calculated on all subjects.
N = number of subjects with available results.
n/% = number/percentage of subjects with concentration ≥ specified value.
95% CI = 95% confidence interval.
GMC = geometric mean antibody concentration.
BMI = body mass index.
Thresholds: 6.2 mIUmL = assay level of detection defining seropositivity 10 mIU/ml = threshold defining seroprotection.

systemic symptoms were fatigue and headache (Table 4). Grade 3 solicited symptoms were reported by 2.9% or fewer of participants in each group. There was no increase in symptoms in either group with consecutive doses. 1.2% or fewer participants in each group sought medical attention for specific local or general symptoms.

Other adverse events occurring within 31 d after each vaccination were reported by 36.1% of participants with DM and 37.2% of controls. Grade 3 adverse events were reported by 8.4% and 6.2% of participants, respectively. Grade 3 events reported by more than one participant in the diabetes group were sinusitis (reported by 1.0% of participants), headache (0.7%), tonsillitis, arthralgia, musculoskeletal pain, and dizziness (each reported by 0.5% of participants). There were no Grade 3 adverse events reported by more than one participant in the control group.

Table 3. Estimated coefficients of the regression analyses on anti-HBs antibody seroprotection status and geometric mean antibody concentrations (GMCs) (according-to-protocol immunogenicity cohort).

		Logistic regression analysis on seroprotection status		Stepwise multiple linear regression analysis on GMCs	
		Odds ratio (95% CI)	p value	GMC ratio (95% CI)	p value
Saturated Model	Presence of diabetes	0.61 (0.36; 1.05)	0.0741	0.47 (0.27; 0.82)	0.0078
	Increased BMI (per 10kg/m^2)	0.52 (0.39; 0.71)	<0.0001	0.33 (0.24; 0.45)	<0.0001
	Female gender	1.15 (0.76; 1.77)	0.5061	1.75 (1.12; 2.72)	0.0136
	Older age (per decade)	0.56 (0.44; 0.70)	<0.0001	0.47 (0.37; 0.58)	<0.0001
	Caucasian ancestry	0.92 (0.45; 1.91)	0.8331	0.62 (0.31; 1.23)	0.1720
	Decreased eGFR	1.00 (0.99; 1.02)	0.5835	1.00 (0.98; 1.01)	0.5694
	Increased HbA1c	1.21 (0.95; 1.53)	0.1206	1.16 (0.94; 1.43)	0.1558
Final model	Increased BMI (per 10kg/m^2)	0.53 (0.40; 0.71)	<0.0001	0.33 (0.24; 0.45)	<0.0001
	Older age (per decade)	0.54 (0.45; 0.65)	<0.0001	0.47 (0.39; 0.56)	<0.0001
	Presence of diabetes	—	—	0.59 (0.37; 0.95)	0.0301
	Female gender	—	—	1.71 (1.10; 2.66)	0.0173

Characteristics were coded in the following order: diabetes group as 1, control group as 0; female as 1, male as 0; Caucasian / European heritage as 1, non-Caucasian / European heritage as 0; age per 10 years; BMI per 10 kg/m^2; eGFR as mL/min/1.73m^2; HbA1c as %. Odds ratio: for binary co variable this represents the ratio of odds between the category coded 1 over the category code 0. For continuous co variable this represents the ratio of odds associated to a co variable increase by one unit. A value above 1 is associated to an increase in seroprotection. GMC ratio: for binary covariables this represents the ratio of GMC between the category coded 1 over the category code 0. For continuous covariables this represents the ratio of GMC associated to a covariable increase by one unit. A value above 1 is associated to an increase in antibody concentration. The p-value for each term tests the null hypothesis that the coefficient is = 0 (no effect). Note: Saturated model is without considering stepwise elimination strategy and final model is after consideration of stepwise elimination strategy.

Table 4. Percentage of participants reporting solicited local and general symptoms within 4 d after vaccination (all doses, total vaccinated cohort).

	Diabetes Group (N = 413)		95 % CI		Control Group (N = 257)		95 % CI	
	n	%	LL	UL	n	%	LL	UL
Solicited local symptom								
Pain	160	38.7	34.0	43.6	115	44.7	38.6	51.1
Redness	84	20.3	16.6	24.5	51	19.8	15.1	25.3
Swelling	49	11.9	8.9	15.4	19	7.4	4.5	11.3
Solicited general symptom								
Fatigue	118	28.6	24.3	33.2	69	26.8	21.5	32.7
Gastrointestinal symptoms	83	20.1	16.3	24.3	47	18.3	13.8	23.6
Headache	97	23.5	19.5	27.9	71	27.6	22.3	33.5
Fever /(Oral) (°C)	15	3.6	2.0	5.9	8	3.1	1.4	6.0

Diabetes Group = Subjects diagnosed with type 2 diabetes within the past 5 y.
Control Group = Subjects with no diagnosis or documented history of diabetes.
N = number of subjects with at least one documented dose.
n/% = number/percentage of subjects reporting the symptom at least once.
95% CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit.

SAEs were reported by 3.8% (95% CI 2.2; 6.2) of participants with diabetes and 1.6% (95% CI 0.4; 3.9) of controls, none of which were considered to be related to vaccination. There was one death during the study: a 45-y old man in the diabetes group was diagnosed with glioblastoma multiforme and died 334 d post-dose 2.

Discussion and conclusions

This prospective, controlled study assessed the immunogenicity and safety of recombinant hepatitis B vaccine in adults with DM versus age and BMI-matched healthy adults. It showed that a 3-dose course of recombinant hepatitis B vaccine induced protective levels of antibodies in 75.4% of diabetic participants and 82.0% control participants matched for age and BMI, with no statistically significant difference in seroprotection rate observed in the ATP cohort. These findings provide immunogenicity and safety data that supports recommendations to vaccinate patients with DM against hepatitis B.

Our study enrolled participants across a wide age range (20–82 years) and BMI (16.7–63.5 kg/m^2). Observed seroprotection rates decreased with age in diabetic patients as well as in control subjects; however, this observation was not confirmed by statistical testing. In addition, compared with previous reports, we observed a lower seroprotection rate in the control group,[15] reflecting the fact that the studied population included a large number of individuals of older age (30% were ≥60 y of age). Immunosenescence associated with older age leads to reduced responses to a range of vaccines, including hepatitis B, tetanus and influenza vaccines.[16-19] Of note, the seroprotection rate in controls who were <40 y of age was 100%.

Increasing age and BMI were associated with decreased likelihood of achieving seroprotection after hepatitis B vaccination in the regression model, with age appearing to be the most clinically relevant factor. Seroprotection rates declined with age in all study participants regardless of DM status, with only 58.2% of participants with DM and 70.2% controls aged ≥60 y achieving seroprotection,

The stepwise regression model suggested that age, BMI, gender and diabetes status all influenced the anti-HBs antibody GMC, indicating that hepatitis B vaccination was less immunogenic in participants with diabetes compared with healthy controls. The clinical relevance of this finding is uncertain in view of the well-established serological correlate of protection for hepatitis B.[20,21] While our study showed an influence of gender on GMC but not on seroprotection rate, other studies of much larger cohorts have previously demonstrated reduced GMCs and seroprotection rates in men compared with women.[22]

The reactogenicity and safety profile of hepatitis B vaccine appeared to be similar in the 2 groups and were consistent with product experience.[15]

While we attempted to recruit individuals broadly representative of the US population, around 50% of participants were recruited from non-US centers. Thus, African Americans were under-represented as compared with the general US population. However, no effect of ancestry on seroprotection was observed, suggesting that this limitation is probably of less clinical relevance. Finally, no data were collected on smoking, which has also been linked to lower responses to hepatitis B vaccination.[23] Another limitation of the study was the sample size recruited, which limited the conclusions which could be drawn. With a larger sample size, the logistic regression model might have shown significance for such characteristics as BMI. Finally, in the stepwise regression as per the p-value, the most influencing factors were age and BMI, followed by treatment group (diabetics) and gender. Considering that the subjects were stratified based on the confounding factors, these differences could be observed between the 2 models. However, this could also be due to random variation occurring in the population considered.

In patients with diabetes aged 20–59 y in whom ACIP recommends vaccination, the seroprotection rate was more than 80% after 3 doses of hepatitis B vaccine. Age appeared to be one of the main drivers of the likelihood to achieve seroprotection. In view of these findings, hepatitis B vaccine should be administered as soon as possible after the diagnosis of DM. Further research in the use of hepatitis B vaccines in persons over 60 y of age may be needed.

Methods

The phase IV study (www.clinicaltrials.gov NCT01627340) was conducted between 24 July 2012 and 18 December 2013. The study was approved by local ethics committees/institutional review boards. Written informed consent was obtained from all

participants before enrolment. The study was conducted in accordance with Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki. Minor deviations from the informed consent procedure were found for 5 participants. Discrepancies between participants' attributes initially recorded in the study databases after study closure resulted in re-allocation of 7 subjects to their correct strata and database correction. As a result, 3 strata included more participants than initially targeted. None of the identified deviations were considered to compromise data integrity, nor led to exclusion of participants from the ATP cohort.

The diabetes group comprised subjects with type-2 DM diagnosed within the past 5 y according to criteria specified by the American Diabetes Association,[24] or who had commenced treatment with any form of anti-diabetic medication within the past 5 y. Participants in the control group had no documented history of DM and had serum glycated hemoglobin (HbA1c) <6.5% (48 mmol/ml IFCC) at the time of screening.

Participants were to be at least 20 y of age at the time of screening and had normal renal function, defined as a glomerular filtration rate (eGFR) ≥50 mL/min per 1.73m^2 as estimated through the Modification of Diet in Renal Disease,[25] or the Chronic Kidney Disease Epidemiology Collaboration equation.[26] Participants were screened for pre-existing HBV surface antigen (HBsAg), anti-HBc antibodies and anti-HBs antibodies, effectively ensuring that only seronegative subjects were included in the primary immunogenicity analysis. Women of childbearing potential practiced adequate contraception from 30 d before initiation until 2 months after completion of the vaccination series, and had a negative pregnancy test at screening and at Visit 1.

Exclusion criteria also included immunosuppression due to any cause, previous hepatitis B vaccination with either a completed or incompleted schedule (based on study staff review of individual immunization records), and advanced heart failure or other clinical conditions that significantly reduced the subject's life expectancy. Subjects were also excluded if they received any vaccine (other than inactivated influenza vaccine) from 30 d before to 30 d after each dose of hepatitis B vaccine, or if they had a history of alcohol or drug abuse in the past 5 y.

The primary objective of the study was to characterize the immunogenicity of the study vaccine in subjects with type-2 DM. Non-diabetic subjects were enrolled as controls and recruited in a way to account for known confounding factors like age and BMI. Since the immunogenicity of the vaccine is well established in non-diabetic subjects, the seroprotection rate was expected to be high in most subgroups of healthy controls, and the study was designed to be purely descriptive (with no confirmatory objectives pre-defined), recruitment was performed with the subjects vs. controls in a 2:1 ratio. Recruitment was further stratified by age (20–39 y, 40–49 y, 50–59 y and ≥60 y) and BMI (<30 kg/m^2; and ≥30 kg/m^2).

To assess anti-HBs seroprotection rates one month after a 3-dose course of recombinant hepatitis B vaccine was chosen as the primary objective because for hepatitis B, there is a well-established correlate of protection i.e. individuals with an anti-HBs antibody titer ≥10mIU/mL are seroprotected.[20,21] Secondary objectives were to assess anti-HBs antibody concentrations in terms of GMCs and to assess the safety and reactogenicity of the study vaccine. The impact of baseline diabetes status, age, BMI, gender, eGFR, ethnicity and HbA1c on the immune response was explored.

The study was conducted in 21 centers in Australia, Canada, New Zealand and the US.

A total of 512 participants with type-2 DM and 256 participants without DM were planned. Taking into account the difficulty enrolling subjects in the diabetes group for some of the strata (youngest age and lowest BMI), it was assumed that these strata would probably remain incompletely enrolled by approximately 20% at the end of the enrolment period: thus providing approximately 408 participants in the diabetes group and approximately 204 matching participants in the control group. Accounting for a 20% drop out rate, enrolment of approximately 326 participants with type-2 DM and approximately 163 controls was anticipated. The objectives of the study were descriptive, and therefore this sample size was not derived based on the power calculations. However, the sample size was calculated to allow the potential for meaningful stratification based on covariables such as BMI, age and the other inclusion criteria parameters. The assumed sample size that could be enrolled in the study was hence calculated in order to ensure that the lower half-width of the 95% CI of the group difference in seroprotection rate remained less than −10% in all considered scenarios.[27]

Screening was conducted 2 to 28 d before the first vaccine dose. Serum creatinine, HBsAg, anti-HBs antibodies, anti-HBc antibodies and HbA1c were measured in all participants at central laboratories using validated commercial assays (Bio Analytical Research Corporation in the US and Australia).

All participants received 3 doses of hepatitis B vaccine at 0, 1 and 6 months. The vaccine (*Engerix*-B^{TM}, GSK Vaccines, Belgium) contained 20 μg of purified recombinant HBsAg adsorbed onto aluminum hydroxide in 1 mL volume, and was administered as an intramuscular injection into the deltoid muscle of the non-dominant arm.

Blood samples were collected at the screening visit and one month after the third vaccine dose. Anti-HBs antibodies were measured with a Chemiluminescence Immunoassay (*Centaur*^{TM}, Siemens Healthcare Diagnostics, Germany) with a level of detection of 6.2 mIU/mL defining seropositivity.

The occurrence of redness, swelling, and pain at the injection site, and fatigue, fever (temperature ≥37.5°C/99.5°F by oral or axillary route), gastrointestinal symptoms and headache that occurred within 4 d (day 0–3) after each dose was recorded on diary cards by participants. Symptoms were graded on a 3-point scale where grade 3 (severe) was defined as redness or swelling >50mm, fever >39.0°C/102.2°F or preventing normal activity for other symptoms. Participants recorded all other adverse events for 30 d after each vaccination (31-d follow up). Serious adverse events (SAEs) and adverse events that resulted in medical attention (defined as hospitalization or an unscheduled visit to/from medical personnel, including emergency room visits) were captured from the first vaccination until one month after the third dose.

Statistical analyses

Analyses were performed using *SAS* (SAS Institute Inc., Cary, NC, United States) software version 9.2 on Statistical Drug Development software and StatXact-8.1.

During the study there were difficulties identifying sufficient number of subjects with type-2 DM who were of younger age (<40 years) and/or lower BMI (<30 kg/m^2). As a result, enrollment in some strata could not be completed. A matching procedure (for age and BMI) was therefore conducted in the ATP analysis to ensure that the allocation of participants with type-2 DM and controls retained a 2:1 ratio in the respective strata.

The primary cohort for the analysis of immune response was the ATP cohort, which included eligible participants who complied with protocol-defined procedures, who had received all 3 doses of hepatitis B vaccine and for whom post-vaccination serology results were available. Control subjects were participants for whom a suitable match was available in the diabetes group. Because >5% of participants were excluded from the ATP immunogenicity cohort, a supplementary analysis was performed on the TVC.

The analysis of safety was performed on the TVC, which included all participants who had received at least one dose of hepatitis B vaccine.

An anti-HBs antibody threshold of 10 mIU/mL defined seroprotection.[20,21] Exploratory group comparisons were performed using the asymptotic standardized 95% CIs for the group difference (Control Group-Diabetes Group) in seroprotection rates.[28] The 95% CI for the adjusted GMC group ratio (Diabetes Group divided by Control Group) was computed using an analysis of variance (ANOVA) model on the logarithm-transformed concentrations, using country, age and BMI strata as fixed effects. The ANOVA model using the general linear model (GLM) procedure without the p-value, was performed on the logarithm10 transformation of the concentrations (pooled variance). We have indeed used the GLM model with ANOVA to assess the GMC ratio between the 2 groups. No adjustments were done on the baseline anti-HBs values.

Regression models used age, BMI, eGFR, and HbA1c as continuous factors, and diabetes, gender and White-Caucasian versus Other ancestry as categorical factors: ancestry was included in the model because of evidence of reduced responses to hepatitis B vaccine in some populations.[29,30] A logistic regression was used to model seroprotection rates and a stepwise multiple linear regression was used to model logarithm-transformed antibody concentrations. The significance level for a variable to stay and to enter in the model was 0.1.

Trademarks

Engerix-B is a trademark of the GSK group of companies.
Centaur is a trademark of Siemens Healthcare Diagnostics.

Abbreviations

ACIP	advisory committee on immunization practices
ANOVA	analysis of variance
Anti-HBc	hepatitis B core antibody
Anti-HBs	hepatitis B surface antibody
ATP	according to protocol
BMI	body mass index
CI	confidence interval
DM	diabetes mellitus
eGFR	glomerular filtration rate
GCP	Good Clinical Practices
GLM	general linear model
GMC	geometric mean concentration
HbA1c	glycated hemoglobin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
SAE	serious adverse events
SAS	statistical analysis software
TVC	total vaccinated cohort

Disclosure of potential conflicts of interest

OVDM, JMM, MDR, PDC, AFT, MDN are employees of the GSK Group of Companies. OVDM, JMM, MDR, MDN hold stock options from the sponsoring company. MD, JTP, and RWS declare no conflict of interest. PR declares having common stock ownership of GSK group of companies. MF receives salary from Colchester Research Group which conducts clinical trials in the area of vaccines, infectious disease and diabetes. MDN was an independent investigator during the recruitment and conduct of this study prior to joining GSK. MDN has received research funding from Abbott Australasia, Baxter, bioCSL, GSK, Novartis, Pfizer, Sanofi, Wyeth, honoraria and speaker's fees from bioCSL, GSK, Novartis, Pfizer, Sanofi, Wyeth for unrelated areas of research (travel medicine, measles-mumps-rubella-varicella vaccine, meningococcal disease, pneumococcal disease, dengue). PRE received research funding from Amgen, GSK, Novartis, Eli-Lilly, Merck, honoraria from Amgen, Takeda, Novo-Nordisk, speakers fee from GSK for unrelated area of research (osteoporosis). RB received research grant from GSK for the study through Medical Research Institute of New Zealand, he has also received personal fee and grant from GSK outside the submitted work.

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Author contributions

OVDM participated in the conception and planning of the study, acquisition of funding, acquisition and extraction of data, coordination between centers, quality checks, interpretation of results and supervision of the study. JTP participated in the acquisition of data and provision of study materials/subjects to the study. MD participated in the acquisition of data, coordination between centers, provision of study materials/subjects and interpretation of results. RB and MF participated in the acquisition and extraction of data, coordination between centers, provision of study materials/subjects and quality checks. PRE participated in the conception and planning of the study, acquisition of data, coordination between centers,

provision of study materials/subjects, interpretation of results and administrative/technical/logistic support. MDN participated in the collection of data and provision of study materials/subjects to the study. PR participated in the recruitment of subjects for the trial. RWS participated in the acquisition and collection of data, coordination between centers and provision of study materials/subjects to the study. MDR participated in the conception and planning of the study and interpretation of the results. PDC performed the statistical analysis. JMM participated in the conception and planning of the study, acquisition of funding, interpretation of the results and supervision of the study. AFT participated in the conception and planning of the study, identification and recruitment of PIs, coordination between centers, interpretation of results, development of investigator meeting materials, administrative/technical/logistical support and supervision of the study. All authors had full access to the data, were involved with developing this manuscript, gave final approval before submission and are accountable for all aspects of the work.

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