Use of heated tobacco products, moderate alcohol drinking, and anti-SARS-CoV-2 IgG antibody titers after BNT162b2 vaccination among Japanese healthcare workers

Shohei Yamamoto [a,*], Akihito Tanaka [b], Norio Ohmagari [c], Koushi Yamaguchi [d], Kazue Ishitsuka [e], Naho Morisaki [e], Masayo Kojima [f], Akihiko Nishikimi [g], Haruhiko Tokuda [h], Manami Inoue [i,j], Shiori Tanaka [i], Jun Umezawa [i], Ryo Okubo [k], Kunihiro Nishimura [l], Maki Konishi [a], Kengo Miyo [m], Tetsuya Mizoue [a]

[a] Department of Epidemiology and Prevention, Center for Clinical Sciences, National Center for Global Health and Medicine, Tokyo, Japan
[b] Department of Laboratory Testing, Center Hospital of the National Center for the Global Health and Medicine, Tokyo, Japan
[c] Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan
[d] Center of Maternal-Fetal, Neonatal and Reproductive Medicine, National Center for Child Health and Development, Tokyo, Japan
[e] Department of social science, National Research Institute for Child Health and Development, Tokyo, Japan
[f] Department of Frailty Research, Research Institute, National Center for Geriatrics and Gerontology, Aichi, Japan
[g] Biosafety Division, Research Institute, National Center for Geriatrics and Gerontology, Aichi, Japan
[h] Bioresource Division, Research Institute, National Center for Geriatrics and Gerontology, Aichi, Japan
[i] Division of Prevention, National Cancer Center Institute for Cancer Control, Tokyo, Japan
[j] Division of Cohort Research, National Cancer Center Institute for Cancer Control, Tokyo, Japan
[k] Clinical Research & Education Promotion Division, National Center of Neurology and Psychiatry, Tokyo, Japan
[l] Department of Preventive Medicine and Epidemiology, National Cerebral and Cardiovascular Center, Osaka, Japan
[m] Center for Medical Informatics Intelligence, National Center for Global Health and Medicine, Tokyo, Japan

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ABSTRACT

The effect of heated tobacco products (HTPs) use and moderate alcohol drinking on immunogenicity to coronavirus disease (COVID-19) vaccines remain elusive. This study aimed to examine the association of tobacco product use and alcohol consumption with anti-SARS-CoV-2 spike IgG antibody titers after the BNT162b2 vaccine. Participants were 3433 healthcare workers receiving two vaccine doses in the 4 national centers for advanced medical and research in Japan. Smoking status and alcohol consumption were assessed via a questionnaire, and anti-SARS-CoV-2 spike IgG titers were measured with chemiluminescent enzyme immunoassay using serum collected on the median of 64 days after the second vaccination. Multilevel linear regression models were used to estimate the geometric mean titers (GMT) and the ratios of means (RoM) between groups with adjustment for covariates. Compared with never-smokers (GMT = 118), IgG antibody titers were significantly lower among HTPs users (including those who also smoked cigarettes) (GMT = 105; RoM = 0.89 [95%CI: 0.78–0.99]) and exclusive cigarettes smokers (GMT = 98; RoM = 0.81 [95%CI: 0.71–0.92]). Compared with non-drinkers of alcohol (GMT = 123), alcohol drinkers consuming <1 *go*/day (GMT = 113; RoM = 0.93 [95%CI: 0.88–0.98]), 1–1.9 *go*/day (GMT = 104; RoM = 0.85 [95%CI: 0.78–0.93]), and ≥ 2 *go*/day (GMT = 103; RoM = 0.84 [95%CI: 0.74–0.96]) had significantly lower antibody titers (*P* for trend<0.01). Spline analysis showed a large reduction of antibody until around 1 *go*/day of alcohol consumption, and then they gradually decreased. Results suggest that in addition to conventional cigarette smoking and heavy alcohol drinking, HTPs use and moderate alcohol drinking may be predictors of lower immunological response to COVID-19 vaccine.

1. Introduction

With increasing coverage of highly effective mRNA vaccines against severe acute respiratory coronavirus 2 (SARS-CoV-2), the burden of coronavirus disease (COVID-19) is expected to decrease. Studies show a large inter-personal variability in vaccine-induced antibody levels (Levin et al., 2021). While numerous factors may underly the variation, it is crucial to clarify modifiable factors that influence post-vaccine immunogenicity. Smoking and heavy alcohol drinking are known to interfere with the activation of innate and acquired immunity (Pasala et al., 2015; Qiu et al., 2017) and may thus suppress vaccine-induced antibody production.

Epidemiological evidence regarding the association of smoking and alcohol use with post-vaccine antibody titers has been inconsistent (Ferrara et al., 2022; Kageyama et al., 2021; Michos et al., 2021; Nomura et al., 2021; Watanabe et al., 2022; Watanabe et al., 2021). No data are available linking post-vaccine antibody response to the use of heated tobacco products (HTPs), which have been increasingly popular in the global market (Caputi et al., 2017). Similar to conventional cigarettes, HTPs contain nicotine (Bekki et al., 2017), which may harm the immune system (Qiu et al., 2017). Regarding alcohol drinking, previous studies did not address the dose-response relationship, with attention to the effect of moderate drinking, which has been hypothesized to enhance vaccine response (Messaoudi et al., 2014). This would be a critical issue for East Asians, including Japanese, who have a high prevalence of the genetic mutation in an alcohol-metabolizing enzyme (Wall and Ehlers, 1995).

The present study sought to examine the association of smoking (including use of HTPs) and alcohol consumption with IgG antibody titers against SARS-CoV-2 spike protein among the staff of national medical research centers in Japan who received two doses of BNT162b2 vaccine.

2. Methods

2.1. Study design

In 2020, a multi-center collaborative study comprising repeat serological surveys was launched among the workers of the six National Centers for Advanced Medical and Research in Japan (6NC). Each NC performs the survey at least once per year during the COVID-19 epidemic. Prior to each survey, the researchers agreed on the survey procedure, including questionnaire and antibody assays used across 6NC, considering the then-current situation of the COVID-19 epidemic, commercial availability of antibody assays, and vaccination program. Written informed consent was obtained from each participant. After completing the opt-out process, the survey data were anonymized and submitted to the study committee for pooled analysis. The study design and procedure for data collection were approved by the ethical committee of each center, while those of the pooling study was approved by that of the National Center for Global Health and Medicine (NCGM) (the approved number: NCGM-G-004233).

2.2. Study population

For the current study, we analyzed data from 4 national centers, which performed a serological survey between June and August 2021 after the completion of the vaccination program for the staff and submitted the data for pooling analysis. The details of the survey and vaccination schedule for each national center were described in Supplemental Table 1.

Of 8190 workers invited to the survey, 5718 participated (70%). Of these, 5013 reported receiving two doses of the COVID-19 vaccine (BNT162b2, Pfizer-BioNTech) by the survey. Of these, we excluded those who attended the survey for the blood draw within 14 days of the second vaccination (*n* = 193), those who had a history of COVID-19 (*n* =

24), those who tested seropositive with an anti-SARS-CoV-2 nucleocapsid protein assay (indicative of the previous infection) (*n* = 7), those who had extremely low spike antibody titers (<1 SU/mL) (*n* = 5), those who lacked data on smoking status or alcohol consumption (*n* = 1339), and those who lacked data on covariates (*n* = 12), leaving 3433 for analysis. In two study centers, where smoking and/or drinking were not asked during the post-vaccination survey, we retrieved these lifestyle data from the pre-vaccination survey. Those who did not participate in the previous survey were excluded from the analysis (*n* = 1337).

2.3. Data collection

We asked participants to donate venous blood and answer a questionnaire including queries on smoking, alcohol consumption, vaccination, the history of COVID-19, and morbid conditions (hypertension, diabetes, and cancer). For smoking conventional cigarettes, we asked participants to select one of the following options: never, quit, smoking less than daily, smoking ≤10, 11–20, or ≥ 21 cigarettes/day. We also asked about the use of new tobacco products (HTPs [IQOS, glo, PULZE, WEECKE, etc.] or e-cigarettes) in the last month. Based on the answers to these questions, we categorized the participants into five groups: never smoker (never smoked cigarette and did not use HTPs), former smoker (quit smoking cigarette and did not use HTPs), current smoker who used only HTPs, current smoker who smoked only conventional cigarettes, and current smoker who used both conventional cigarettes and HTPs (dual users). We did not consider e-cigarette use because the prevalence of e-cigarettes was low in Japan (Sugiyama and Tabuchi, 2020). In post hoc analysis, we combined the categories of exclusive HTPs users and dual users in one to increase statistical power.

For alcohol consumption, we asked about the frequency of alcohol intake (ranging from never to daily) and the amount of intake per occasion (ranging from <0.5 to ≥4 *go*/day). In Japan, *go* (180 mL) is used as the conventional unit to measure alcohol amount; 1 *go* of Japanese *sake* contains approximately 23 g of ethanol, which is equivalent to 500 mL of beer, 110 mL of shochu (25% alcohol content), double (60 mL) of whisky, or 180 mL of wine. We calculated averaged daily consumption and classified the participants into five groups: non-drinker, occasional drinker (1–3 days/month), and weekly drinker consuming <1 *go*/day, 1–1.9 *go*/day, or ≥ 2 *go*/day.

2.4. Serological assay

We quantitatively measured SARS-CoV-2 IgG antibodies against spike protein in serum with the chemiluminescence enzyme immunoassay (CLEIA) platform (HISCL) manufactured by Sysmex Co. (Kobe, Japan) to assess vaccine-induced antibody response. A concentration of ≥10 SU/ml was considered seropositive (Noda et al., 2021). We confirmed that spike IgG antibody titers measured with this assay were highly correlated with those measured with the AdviseDx SARS-CoV-2 IgG II assay (Abbott ARCHITECT®) using data of two centers that measured antibodies with both assays (*n* = 2961): spearman's rho = 0.96 (95% confidence intervals [CI]: 0.95–0.96) (Supplemental Fig. 1).

We also quantitatively tested SARS-CoV-2 IgG antibodies against nucleocapsid protein with the HISCL platform to assess past exposure of participants to SARS-CoV-2. A concentration of ≥10 SU/ml was considered seropositive. The sensitivity and specificity were 100% and 99.8%, respectively (Noda et al., 2021).

2.5. Statistical analysis

We performed multilevel linear regression analysis to examine the associations of smoking and alcohol use with anti-spike IgG antibody titers on log-scale. Fixed-effect covariates included age (continuous), sex, body mass index (BMI) (<18.5, 18.5–24.9, 25.0–29.9, and ≥ 30 kg/m^2), the interval between the second vaccination and blood sampling (continuous), and a square term of the interval, morbid conditions

(diabetes, hypertension, and cancer), smoking status, and alcohol consumption. The random-effects intercept term was set by the national center level. The estimated effects of exposures were back-transformed to present the geometric mean titer (GMT) and ratios of means. We tested the statistical significance between groups by comparing the ratios of means. We drew a restricted cubic spline curve to visualize the dose-response association between alcohol consumption and IgG antibody titers, with 3 knots placed at the 5th, 50th, and 95th percentiles as recommended (Desquilbet and Mariotti, 2010). Statistical analysis was performed using Stata 17.0 (StataCorp LLC). All P values were 2-sided, and $P < 0.05$ was considered statistically significant.

3. Results

The median age of participants was 41 years (interquartile [IQR]: 30–50 years), 72% were women, 3% were obese (BMI $\geq$ 30 kg/m^2), and 1–8% had morbid conditions (8% for hypertension, 2% for diabetes, 1% for cancer). One-third were nurses (34%), followed by allied health professionals (18%), administrative staff (15%), doctors (14%), and researchers (13%). Of 211 current smokers (6.1% of the participants), nearly half (54%) used HTPs. Thirty-nine percent drank alcoholic beverages at least once per week. The median interval between the second vaccination and blood sampling was 64 days (IQR, 38–74 days). The median titers of anti-SRAS-CoV-2 spike IgG antibody were 137 SU/mL (IQR: 74–243), with 99.5% (3416/3433) of participants were seropositive (threshold $\geq$10 SU/mL) (Table 1).

Current smokers using any tobacco product had lower antibody titers (GMT, 101; ratio of mean, 0.85 [95% CI: 0.78–0.93]) compared with never-smokers (supplemental Table 2) after adjustment of age, sex, BMI, the interval between the second vaccination and blood sampling, morbidities, and alcohol consumption. As shown in Table 2, exclusive cigarette smokers had significantly lower GMT than never-smokers (adjusted GMT, 118 versus 96; ratio of means, 0.81 [95%CI: 0.71–0.92]). Exclusive HTPs users and dual users also showed similarly lowered adjusted GMT (103 and 107, respectively), although the differences from never-smokers were not statistically significant (ratio of means, 0.87 [95%CI: 0.75–1.02] and 0.90 [95%CI: 0.75–1.07], respectively). In the post-hoc analysis combining the two categories of HTPs users ($n = 113$), the reduction reached statistical significance (adjusted GMT, 105; ratio of mean, 0.89 [95%CI: 0.78–0.99]). Regarding the number of conventional cigarettes smoked, those consuming $\geq$11 cigarettes per day showed a greater reduction in IgG titers than those consuming <11 cigarettes per day; adjusted GMTs (ratio of means) were 92 (0.78 [95%CI: 0.63–0.96]) and 103 (0.87 [95% CI: 0.75–1.00]), respectively (Supplemental Table 2).

Weekly drinkers of alcoholic beverages had significantly lower antibody titers than non-drinkers (Supplemental Table 2) with adjustment for covariates. Spike IgG antibody titers steadily decreased with an increasing amount of alcohol consumption. The adjusted GMTs of the antibody for non-drinkers, occasional drinkers, and weekly drinkers consuming <1 *go*/day, 1–1.9 *go*/day, and $\geq$ 2 *go*/day were 123 (95%CI: 98–154), 118 (95%CI: 94–148), 113 (95%CI: 90–143), 104 (95%CI: 82–132), and 103 (95%CI: 80–134), respectively (P for trend <0.01) (Table 2). Compared with non-drinkers, antibody titers were statistically significantly lower even among moderate drinkers consuming <1 *go*/day (<23 g ethanol), with the ratio of means for 0.93 (95%CI: 0.88–0.98). Spline analysis yielded a clear dose-response relationship, showing a large reduction in the ratio of means until around 1 *go*/day of alcohol consumption, followed by a gradual decreasing trend with increasing alcohol consumption (Fig. 1).

4. Discussion

In 3433 staff of national medical research centers in Japan who completed two-dose of the COVID-19 BNT162b2 vaccine, HTPs users (exclusive or combined with conventional cigarettes) and exclusive

Table 1
Characteristics of participants according to national center.

Characteristic	Total	National center			
		NCC	NCCHD	NCGG	NCGM
	N = 3433	N = 470	N = 628	N = 483	N = 1852
Smoking status, n(%)					
Never-smoker	2829 (82)	383 (81)	542 (86)	396 (82)	1518 (81)
Past smoker	393 (11)	73 (16)	63 (10)	50 (10)	209 (11)
Current smoker	211 (6)	14 (3)	23 (4)	38 (8)	136 (7)
Exclusive HTPs user	62 (2)	7 (1)	9 (1)	5 (1)	41 (2)
Dual user of HTPs and cigarettes	51 (1)	1 (0.2)	2 (0.3)	17 (4)	31 (2)
Exclusive cigarette smoker	98 (3)	6 (1)	12 (2)	16 (3)	64 (3)
Alcohol consumption, n (%)					
Non-drinker	1323 (39)	161 (34)	250 (40)	208 (43)	704 (38)
Occasional drinker	780 (23)	113 (24)	156 (25)	109 (23)	402 (22)
Weekly drinker	1330 (39)	196 (42)	222 (35)	166 (34)	746 (40)
<1 *go*/day	962 (28)	160 (34)	171 (27)	114 (24)	517 (28)
1 to 1.9 *go*/day	274 (8)	29 (6)	40 (6)	34 (7)	171 (9)
$\geq$2 *go*/day	94 (3)	7 (1)	11 (2)	18 (4)	58 (3)
Female sex, n(%)	2481 (72)	358 (76)	496 (79)	318 (66)	1309 (71)
Median age, year (IQR)	41 (30–50)	44 (35–51)	41 (31–49)	41 (30–50)	39 (29–49)
Median interval between the second dose of vaccine and antibody test, days (IQR)	64 (38–74)	56 (31–63)	103 (103–103)	33 (29–35)	66 (54–69)
Job category, n(%)					
Doctor	495 (14)	49 (10)	143 (23)	42 (9)	261 (14)
Nurse	1171 (34)	84 (18)	209 (33)	166 (34)	712 (38)
Allied health professional	628 (18)	94 (20)	84 (13)	152 (31)	298 (16)
Administrative staff	510 (15)	105 (22)	85 (14)	75 (15)	245 (13)
Researcher	457 (13)	130 (28)	86 (14)	22 (5)	219 (12)
Other	172 (5)	8 (2)	21 (3)	26 (5)	117 (6)
Body mass index, n (%)					
<18.5 kg/m^2	377 (11)	54 (11)	66 (11)	44 (9)	213 (12)
18.5 to 24.9 kg/m^2	2533 (74)	334 (71)	481 (77)	366 (76)	1352 (73)
25 to 29.9 kg/m^2	432 (13)	71 (15)	64 (10)	65 (13)	232 (13)
$\geq$30 kg/m^2	91 (3)	11 (3)	17 (3)	8 (2)	55 (3)
Chronic diseases, n (%)					
Hypertension	273 (8)	41 (9)	60 (10)	40 (8)	132 (7)
Diabetes	76 (2)	9 (2)	12 (2)	14 (3)	41 (2)
Cancer	48 (1)	11 (2)	12 (2)	3 (1)	22 (1)

(*continued on next page*)

Table 1 (*continued*)

Characteristic	Total	National center			
		NCC	NCCHD	NCGG	NCGM
	$N = 3433$	$N = 470$	$N = 628$	$N = 483$	$N = 1852$
Seropositivity against the SARS-CoV-2 spike protein, n(%)	3440 (99.5)	477 (99.6)	620 (98.3)	484 (100.0)	1859 (99.8)

Data are presented as median (IQR) for continuous measures and n (%) for categorical measures.

HTPs: heated tobacco products, IQR: interquartile range, NCC: National Cancer Center, NCCHD: National Center for Child Health and Development, NCGG: National Center for Geriatrics and Gerontology, NCGM: National Center for Global Health and Medicine, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

Table 2

The estimated geometric means (GMT) with 95% confidence intervals (CI) with smoking status and alcohol consumption among 3433 vaccinated healthcare workers.

Variables	No.	SARS-CoV-2 spike IgG antibodies		P-value
		Estimated GMT (95% CI)	Ratio of means (95% CI)	
Smoking status				
Never-smoker	2829	118 (94–149)	Reference	Reference
Past smoker	393	114 (90–144)	0.96 (0.90–1.03)	0.28
Exclusive HTPs user	62	103 (79–136)	0.87 (0.75–1.02)	0.09
Dual user of HTPs and conventional cigarettes	51	107 (80–142)	0.90 (0.75–1.07)	0.24
HTPs user (exclusive and dual users)[a]	*113*	*105 (81–135)*	*0.89 (0.78–0.99)*	*0.04*
Exclusive conventional cigarettes smoker	98	96 (74–124)	0.81 (0.71–0.92)	<0.01
Alcohol consumption				
Non-drinker	1323	123 (98–154)	Reference	Reference
Occasional drinker	780	118 (94–148)	0.96 (0.91–1.02)	0.16
<1 *go*/day	962	113 (90–143)	0.93 (0.88–0.98)	<0.01
1–1.9 *go*/day	274	104 (82–132)	0.85 (0.78–0.93)	<0.01
≥2 *go*/day	94	103 (80–134)	0.84 (0.74–0.96)	0.01
			P for trend[b] <0.01	

The geometric mean titers of anti-SARS-CoV-2 spike IgG antibodies were estimated using the multilevel linear regression model accounting for each national center as the group factor. The model was adjusted for age (years, continuous), sex, body mass index (<18.5, 18.5–24.9, 25.0–29.9, and ≥ 30 kg/m^2), the interval between the second vaccination and blood sampling (days, continuous), the squared term of the interval, and morbid conditions (diabetes, hypertension, and cancer), with mutual adjustment for smoking status and alcohol consumption.

GMT: geometric mean titers, HTPs: heated tobacco products, IgG: Immunoglobulin G, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, 95% CI: 95% confidence intervals.

[a] Post-hoc analysis combining the two categories of HTPs users.

[b] Based on the multilevel linear regression model incorporating the category of alcohol consumption as a continuous variable.

cigarettes smokers had significantly lower anti-SARS-CoV-2 spike IgG antibody titers compared with never-smokers. There was a clear decreasing trend of antibody titers with increasing alcohol consumption,

with significant reduction being observed even at a modest amount of alcohol.

The present finding of lower antibody titers among cigarette smokers agrees with those of previous studies (Ferrara et al., 2022; Michos et al., 2021; Nomura et al., 2021; Watanabe et al., 2021) and is also consistent with the mechanism for smoking-mediated immunopathology. Cigarette smoking induces chronic inflammation and downregulates CD4+ T cells and B cells, which are responsible for producing antigen-specific antibodies (Sopori, 2002). Analyses of immunoglobulins confirmed a decreased production of IgA, IgG, and IgM in cigarettes smokers (Qiu et al., 2017).

To our knowledge, the present study is the first to show post-vaccination antibody titers in relation to HTPs. We observed a decrease of antibody titers among HTPs users relative to never smokers, although the reduction was less than that associated with cigarette smoking. This could be ascribed to nicotine levels of HTPs aerosol are comparable to those released by conventional cigarettes (Bekki et al., 2017; Simonavicius et al., 2019). Experimental studies showed that chronic exposure to nicotine inhibited antibody-forming cell response, impaired antigen-mediated signaling in T cells, and induced T cell anergy (Sopori, 2002). The present finding, together with experimental data, adds evidence to support the adverse effect of HTPs use against immunogenicity to the COVID-19 vaccination.

We found lower titers of anti-SARS-CoV-2 spike IgG antibodies among weekly drinkers of alcoholic beverages, a finding consistent with previous studies (Kageyama et al., 2021). The novelty of the present study is to show a clear dose-response relationship; antibody titers steadily decreased with increasing alcohol consumption, with significant reduction being observed even at a moderate amount of alcohol. This finding is at variance with the hypothesis derived from mechanistic inference; moderate alcohol drinking can boost antibody response by decreasing the level of pathological inflammation and increasing circulating immunoglobulins and plasma antioxidants levels (Messaoudi et al., 2014). However, our observation is compatible with animal experiments showing that low doses of alcohol inhibited antigen-stimulated B-cells proliferation and their antibody production (Aldo-Benson, 1989; Aldo-Benson et al., 1992). The present finding, together with experimental data, indicates that the detrimental effects of alcohol intake on the COVID-19 immunogenicity may start at the low doses without a threshold.

Strengths of the present multi-center study include a large sample size, quantitative assessment of alcohol intake, information on the type of tobacco products, and the adjustment of multiple potential confounders. We should also acknowledge study limitations. First, as this study is cross-sectional, we cannot determine whether smoking and alcohol use were associated with the surge of antibodies in response to vaccination or the decay of antibodies over time. Second, the number of exclusive HTPs users was not large enough to detect the reduction of IgG titers among them with statistical significance. Nonetheless, the point estimate of exclusive HTPs users was similar to those of dual users and exclusive conventional cigarettes users, supporting the detrimental role of HTPs exclusive use on the immune response to the COVID-19 vaccine. Third, we did not assess cellular immune response, another key mechanism of infection protection (Tan et al., 2021). Fourth, we did not measure neutralizing antibodies, a more reliable marker of humoral immune response. Nevertheless, spike IgG antibody titers measured with the assay we employed are known to well correlate with neutralizing antibody titers (spearman's $\rho = 0.71$, 95% CI: 0.63–0.77) (Maeda et al., 2021). Fifth, we cannot rule out the possibility of residual confounding. For example, as a measure of obesity, we adjusted for BMI, which was less correlated with anti-SARS-CoV-2 spike antibody titers than waist circumference (Malavazos et al., 2022). Sixth, the present participants were mainly healthy individuals, with only 3% being obese and 1–8% having morbid conditions. In a study in Caucasian patients with morbid obesity, smoking was not associated with additional adverse effects on the immunogenicity to the COVID-19 vaccine
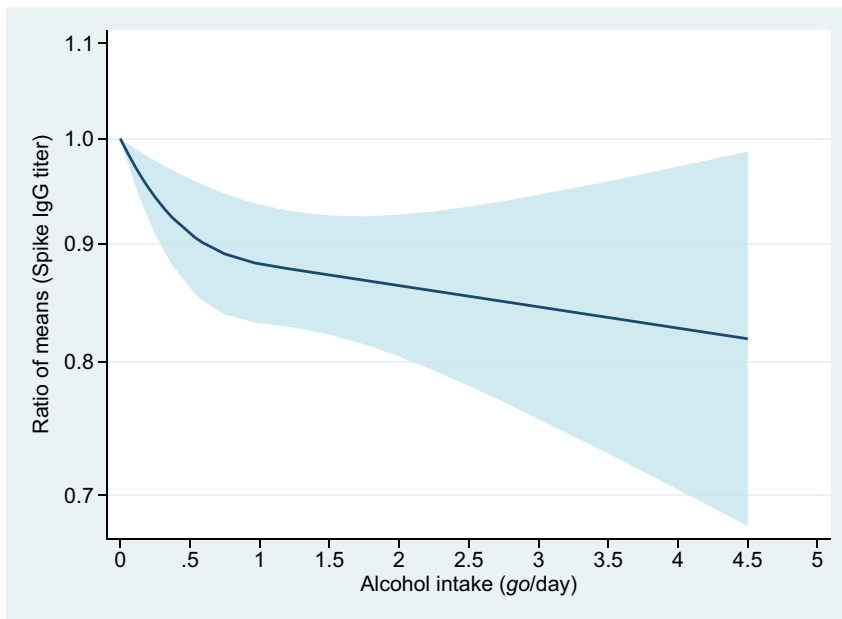
Fig. 1. Dose-response association between alcohol consumption and anti-SARS-CoV-2 spike IgG titers.
The y-axis is the ratio of means with the shaded area representing 95% confidence intervals (linear trend, $P < 0.01$; non-linear trend, $P = 0.02$). The reference value is 0 *go*/day (non-drinkers). The model was adjusted for age (years, continuous), sex, body mass index (<18.5, 18.5–24.9, 25.0–29.9, and ≥ 30 kg/m^2), the interval between the second vaccination and blood sampling (days, continuous), the squared term of the interval, morbid conditions (diabetes, hypertension, and cancer), and smoking status.
One *go* of Japanese sake contains approximately 23 g of ethanol.

(Watanabe et al., 2022), suggesting the effects of smoking may differ according to participants' background. Caution should be exercised in generalizing our results to populations with other ethnicities and with morbidities. Finally, many East Asians are genetically deficient in one of the enzymes involved in metabolizing alcohol (aldehyde dehydrogenase) (Wall and Ehlers, 1995); thus, the present finding regarding alcohol use may not be observed in populations with different genetic backgrounds.

5. Conclusion

Cigarettes smokers and, to a lesser extent, HTPs users had lower IgG antibody titers against SAR-CoV-2 spike protein after vaccination, relative to never-smokers. The antibody titers steadily decreased with increasing alcohol consumption, with significant reduction being observed even at moderate amounts of alcohol. In addition to conventional cigarette smoking and heavy alcohol use, the use of HTPs and moderate alcohol drinking may deteriorate immune response to COVID-19 vaccine.

Ethics approval

The study design and procedure for data collection were approved by the ethical committee of each center, while those of pooling study were approved by that of the National Center for Global Health and Medicine (NCGM) (the approved number: NCGM-G-004233).

Consent to participate

Written informed consent was obtained from each participant. After completing the opt-out process, the survey data were anonymized and submitted to the study committee for pooled analysis.

Data availability

The data underlying this article cannot be shared publicly due to ethical restrictions and participant confidentiality concerns, but de-identified data are available from Dr. Mizoue (Department of Epidemiology and Prevention, Center for Clinical Sciences, National Center for Global Health and Medicine, Tokyo, Japan) to qualified researchers on reasonable request.

CRediT authorship contribution statement

Shohei Yamamoto: Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Akihito Tanaka:** Investigation, Resources, Writing – review & editing. **Norio Ohmagari:** Writing – review & editing, Supervision. **Koushi Yamaguchi:** Methodology, Investigation, Writing – review & editing, Supervision. **Kazue Ishitsuka:** Investigation. **Naho Morisaki:** Investigation, Writing – review & editing. **Masayo Kojima:** Methodology, Investigation, Writing – review & editing, Supervision. **Akihiko Nishikimi:** Investigation, Writing – review & editing. **Haruhiko Tokuda:** Investigation, Writing – review & editing. **Manami Inoue:** Methodology, Investigation, Writing – review & editing, Supervision. **Shiori Tanaka:** Investigation, Writing – review & editing. **Jun Umezawa:** Investigation, Writing – review & editing. **Ryo Okubo:** Methodology, Investigation, Supervision. **Kunihiro Nishimura:** Methodology, Investigation, Writing – review & editing, Supervision. **Maki Konishi:** Investigation, Data curation, Writing – review & editing. **Kengo Miyo:** Resources, Writing – review & editing. **Tetsuya Mizoue:** Conceptualization, Methodology, Software, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

and administrative support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ypmed.2022.107123.

References

Aldo-Benson, M., 1989. Mechanisms of alcohol-induced suppression of B-cell response. Alcohol. Clin. Exp. Res. 13, 469–475.

Aldo-Benson, M., Kluve-Beckerman, B., Hardwick, J., Lockwood, M., 1992. Ethanol inhibits production of messenger ribonucleic acid for kappa-chain in stimulated B lymphocytes. J. Lab. Clin. Med. 119, 32–37.

Bekki, K., Inaba, Y., Uchiyama, S., Kunugita, N., 2017. Comparison of Chemicals in Mainstream Smoke in heat-not-burn tobacco and combustion cigarettes. J. UOEH 39, 201–207.

Caputi, T.L., Leas, E., Dredze, M., Cohen, J.E., Ayers, J.W., 2017. They're heating up: internet search query trends reveal significant public interest in heat-not-burn tobacco products. PLoS One 12, e0185735.

Desquilbet, L., Mariotti, F., 2010. Dose-response analyses using restricted cubic spline functions in public health research. Stat. Med. 1037–1057.

Ferrara, P., Ponticelli, D., Agüero, F., Caci, G., Vitale, A., Borrelli, M., Schiavone, B., Antonazzo, I.C., Mantovani, L.G., et al., 2022. Does smoking have an impact on the immunological response to COVID-19 vaccines? Evidence from the VASCO study and need for further studies. Public Health 203, 97–99.

Kageyama, T., Ikeda, K., Tanaka, S., Taniguchi, T., Igari, H., Onouchi, Y., Kaneda, A., Matsushita, K., Hanaoka, H., et al., 2021. Antibody responses to BNT162b2 mRNA COVID-19 vaccine and their predictors among healthcare workers in a tertiary referral hospital in Japan. Clin. Microbiol. Infect. 27 (12), 1861.e1–1861.e5.

Levin, E.G., Lustig, Y., Cohen, C., Fluss, R., Indenbaum, V., Amit, S., Doolman, R., Asraf, K., Mendelson, E., et al., 2021. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. N. Engl. J. Med. 385, e84.

Maeda, K., Amano, M., Uemura, Y., Tsuchiya, K., Matsushima, T., Noda, K., Shimizu, Y., Fujiwara, A., Takamatsu, Y., et al., 2021. Correlates of neutralizing/SARS-CoV-2-S1-binding antibody response with adverse effects and immune kinetics in BNT162b2-vaccinated individuals. Sci. Rep. 11, 22848.

Malavazos, A.E., Basilico, S., Iacobellis, G., Milani, V., Cardani, R., Boniardi, F., Dubini, C., Prandoni, I., Capitanio, G., et al., 2022. Antibody responses to BNT162b2 mRNA vaccine: infection-naïve individuals with abdominal obesity warrant attention. Obesity 30, 606–613.

Messaoudi, I., Pasala, S., Grant, K., 2014. Could moderate alcohol intake be recommended to improve vaccine responses? Exp. Rev. Vacc. 13, 817–819.

Michos, A., Tatsi, E.-B., Filippatos, F., Dellis, C., Koukou, D., Efthymiou, V., Kastrinelli, E., Mantzou, A., Syriopoulou, V., 2021. Association of total and neutralizing SARS-CoV-2 spike -receptor binding domain antibodies with epidemiological and clinical characteristics after immunization with the 1st and 2nd doses of the BNT162b2 vaccine. Vaccine 39, 5963–5967.

Noda, K., Matsuda, K., Yagishita, S., Maeda, K., Akiyama, Y., Terada-Hirashima, J., Matsushita, H., Iwata, S., Yamashita, K., et al., 2021. A novel highly quantitative and reproducible assay for the detection of anti-SARS-CoV-2 IgG and IgM antibodies. Sci. Rep. 11.

Nomura, Y., Sawahata, M., Nakamura, Y., Kurihara, M., Koike, R., Katsube, O., Hagiwara, K., Niho, S., Masuda, N., et al., 2021. Age and smoking predict antibody Titres at 3 months after the second dose of the BNT162b2 COVID-19 vaccine. Vaccines 9, 1042.

Pasala, S., Barr, T., Messaoudi, I., 2015. Impact of alcohol abuse on the adaptive immune system. Alcohol Res. 37, 185–197.

Qiu, F., Liang, C.-L., Liu, H., Zeng, Y.-Q., Hou, S., Huang, S., Lai, X., Dai, Z., 2017. Impacts of cigarette smoking on immune responsiveness: up and down or upside down? Oncotarget 8, 268–284.

Simonavicius, E., McNeill, A., Shahab, L., Brose, L.S., 2019. Heat-not-burn tobacco products: a systematic literature review. Tob. Control. 28, 582–594.

Sopori, M., 2002. Effects of cigarette smoke on the immune system. Nat. Rev. Immunol. 2, 372–377.

Sugiyama, T., Tabuchi, T., 2020. Use of multiple tobacco and tobacco-like products including heated tobacco and E-cigarettes in Japan: a cross-sectional assessment of the 2017 JASTIS study. Int. J. Environ. Res. Public Health 17, 2161.

Tan, A.T., Linster, M., Tan, C.W., Le Bert, N., Chia, W.N., Kunasegaran, K., Zhuang, Y., Tham, C.Y.L., Chia, A., et al., 2021. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell Rep. 34, 108728.

Wall, T.L., Ehlers, C.L., 1995. Genetic influences affecting alcohol use among Asians. Alcohol Health Res. World 19, 184–189.

Watanabe, M., Balena, A., Tuccinardi, D., Tozzi, R., Risi, R., Masi, D., Caputi, A., Rossetti, R., Spoltore, M.E., et al., 2021. Central obesity, smoking habit, and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine. Diabetes Metab. Res. Rev. 38 (1January 2022e3465).

Watanabe, M., Balena, A., Masi, D., Tozzi, R., Risi, R., Caputi, A., Rossetti, R., Spoltore, M.E., Biagi, F., et al., 2022. Rapid weight loss, central obesity improvement and blood glucose reduction are associated with a stronger adaptive immune response following COVID-19 mRNA vaccine. Vaccines 10, 79.