Factors associated with anti-SARS-CoV-2 antibody titres 3 months post-vaccination with the second dose of BNT162b2 vaccine: a longitudinal observational cohort study in western Greece

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ABSTRACT

Objectives Vaccination against SARS-CoV-2 has been extensively deployed during COVID-19 pandemic. One efficient method to evaluate response to vaccination is the assessment of humoral immunity by measuring SARS-CoV-2 antibody titres. We investigated the association between anthropometric parameters (age, body mass index), smoking, diabetes, statin use, hypertension, levels of 25(OH)D and dehydroepiandrosterone sulfate (DHEAS), and SARS-CoV-2 antibody titres after vaccination.

Design In this longitudinal observational cohort study, 712 subjects were tested for SARS-CoV-2 antibodies 3 months after the second dose of BNT162b2 vaccine. Multiple linear regression analysis was performed to identify which factors are associated with the antibody titres.

Setting Healthcare units of western Greece (University Hospital of Patras and “St Andrews” State General Hospital of Patras).

Participants All adults receiving their second dose of BNT162b2 vaccine at the participating healthcare units were eligible to participate in the study. Exclusion criteria were SARS-CoV-2 infection or positive SARS-CoV-2 antibody titre at baseline. Patients who did not provide all necessary information were excluded from our analyses.

Results We found age to be negatively associated with antibody titre (−0.005; 95% CI −0.009 to −0.001, p=0.0073), as was male gender (−0.11; 95% CI −0.1738 to −0.04617, p=0.0008). The interaction of age and gender was significant (−0.01090; 95% CI −0.01631 to −0.005490, p=0.0001), highlighting that the rate of decline in antibody titre with increasing age tends to be higher in men rather than in women. No linear trend was found between DHEAS levels and antibody titres when the lower quartile of DHEAS levels was used as reference. Tobacco use was associated with low antibody titre (−0.1097; 95% CI −0.174 to −0.046, p=0.0008) but overweight, obese or underweight subjects had similar antibody responses to normal-weight individuals. Although subjects with diabetes and hypertension had numerically lower antibody titres, this association was not statistically significant. Vitamin D levels showed no clear relationships with antibody titres.

Conclusions Age, male gender and tobacco use are negatively associated with antibody titres after COVID-19 vaccination, but our data showed no clear correlation with vitamin D levels.

Trial registration number NCT04954651; Results.

INTRODUCTION

SARS-CoV-2 has spread worldwide since January 2020 resulting by the end of July 2021 to about 197 million confirmed cases of COVID-19 with over 4 million deaths according to WHO. Pharmaceutical and biotechnology companies started developing COVID-19 vaccines and four have received so far authorisation to be used in the European Union (BioNTech and Pfizer, Moderna, AstraZeneca, Johnson & Johnson/Janssen Pharmaceuticals). BNT162b2 vaccine developed by BioNTech and Pfizer is a lipid
nanoparticle-formulated nucleoside-modified RNA encoding the SARS-CoV-2 spike glycoprotein and was the first to receive approval for use in the European Union, hence being the first to be administered in Greece. The safety and 95% efficacy in preventing symptomatic COVID-19 of BNT162b2 vaccine had been initially shown by the BioNTech-Pfizer clinical trials and similar results were obtained in other studies.2-5

The humoral immune response to COVID-19 vaccination has usually been assessed in clinical trials by measuring the antibody titre (including IgG) against the SARS-CoV-2 spike (S) protein.6,7 However, Food and Drug Administration (FDA) and other regulatory bodies do not recommend assessment of immunity after COVID-19 vaccination by measuring antibody titre. Nevertheless, commercial serological assays show over 90% positive agreement for distinguishing neutralising antibodies at the same neutralising titre, while the negative percent agreement was poorer (less than 80%).8 Hence, antibody titre against SARS-CoV-2 spike (S) protein can be an easy and cost-effective measure of the humoral immune response to COVID-19 vaccination in the real world.

It has been a matter of concern that specific populations will not be able to develop an effective immune response after COVID-19 vaccination. Such groups include but they are not limited to aged individuals, immunocompromised patients due to autoimmune diseases or due to immunosuppressive medication, patients with cancer and obese/patients with diabetes. Thus, studies with kidney transplant recipients,9 patients with cancer10-11 came into focus. Moreover, the identification of other factors that can possibly impair or boost the immune response is important so as to identify individuals that might be at risk even after COVID-19 vaccination. Such factors can be obesity,12 ageing,13 dehydroepiandrosterone sulfate (DHEAS), a steroid that declines with ageing,14 vitamin D,15 statin use,16 smoking17 and hypertension.18

As previous studies on SARS-CoV-2 antibodies response (reviewed in the literature19) are limited by the number of patients and their methodologies, we report herein the real-world humoral immunogenic response to BNT162b2 vaccine in subjects of western Greece vaccinated against COVID-19, and we assess potential parameters that may affect this response.

METHODS
Design, setting and study population
This is a longitudinal observational cohort study (ClinicalTrials.gov Identifier: NCT04954651, approved by the University Hospital of Patras Ethics Committee, approval ID 99-25/2/202) in healthcare units of western Greece (University Hospital of Patras and “St Andrews” State General Hospital of Patras). Blood samples were drawn before vaccination, 3 weeks and 3 months after the second dose of vaccination with BNT162b2 vaccine (BioNTech and Pfizer) as part of the national COVID-19 vaccination programme in Greece. Blood samples at the 3 weeks timepoint were drawn only from a portion of our cohort (figure 1A). Participation was based on informed written consent. All adults of any age and gender were eligible to participate in this study. Data about body weight, height, smoking, diabetes, hypertension and statin use were obtained from all participants based on questionnaires. Exclusion criteria were medical history of COVID-19 or infection with SARS-CoV-2 during the study or positive SARS-CoV-2 spike (S) protein antibody titre at baseline (before vaccination). Moreover, patients who did not provide all necessary information in the questionnaire or there was not enough sample to run all the assays. (B) SARS-CoV-2 antibody titre from 3 weeks to 3 months post-vaccination with BNT162b2. Dots indicate individual values (n=198) and straight line indicates means of log antibody titre. *p<0.0001, paired t-test.
from Free Statistics Calculator V.4.0 by Daniel Soper (Fullerton, California, USA). Specifically, anticipated effect size was set to 0.02 (which is considered small), desired statistical power level to 0.8, number of predictors to 9 and p value to 0.05, resulting to a minimum required sample size: 788.

Laboratory measurements
Serum samples were stored in a −20°C freezer. All serum sample measurements were performed in a Cobas 6000 E601 analyser (Roche Diagnostics GmbH, Mannheim, Germany) using electrochemiluminescence (ECL) technology for immunoassay analysis. Quantitative determination of antibodies (including IgG) to SARS-CoV-2 spike (S) protein receptor binding domain (RBD) was performed using the Elecsys Anti-SARS-CoV-2 S immunoassay (Roche). Antibody levels higher than 0.8 U/L were considered positive (measurement range 0.4–250 U/mL). Values higher than 250 U/mL were diluted 1:20 to obtain the exact value. 25(OH)D (vitamin D) and DHEAS levels were assessed at the 3 months post-vaccination timepoint. The measuring range for DHEAS is 0.2–1000 ng/dL and for vitamin D is 3.00–120 ng/mL, respectively.

Statistical analysis
Descriptive statistics for baseline characteristics of the study population were calculated as means±SD. To assess which factors affect the SARS-CoV-2 antibody titre, multiple linear regression modelling was performed with log-transformed antibody titre as a continuous dependent variable and by stepwise backward elimination using p<0.05 as the threshold for removing non-significant independent variables. Age, body mass index (BMI), DHEAS and vitamin D were checked for linear association with the log-transformed antibody titre, and if no linear trend was found for a variable, this variable was categorised using quartiles (DHEAS and vitamin D) or pre-existing categories for BMI (underweight; <18.5, normal weight; 18.5–24.9, overweight; 25–29.9, obesity; >30). Participants who did not answer all of the questions from the questionnaire were excluded from the analysis. GraphPad Prism V.9.0.0 for Windows (GraphPad Software, San Diego, California, USA) was used for all the statistical analyses and graphs generation. The graphs in figure 2A,B were generated using Microsoft Excel V.365 (Microsoft, Redmond, Washington, USA).

Patient and public involvement
All subjects involved in this study were informed about the possibility to participate in this study during their vaccination. No subjects were involved in the development of the research question, in the design, recruitment and conduct of the study. The results of this study will be summarised and translated in the patients’ native language (mainly Greek) and will be posted as a poster in the healthcare units involved and sent in all patients who provided their email address.

RESULTS

Characteristics of the study sample
All 929 participants in the present study were screened at baseline for the presence of SARS-CoV-2 antibodies. Four were excluded due to being tested positive for SARS-CoV-2 antibodies at baseline. Further four participants suffered from COVID-19 during the study and were also excluded (figure 1A). Two hundred nine participants were also excluded because at least one parameter in the questionnaire was not answered (for instance, not giving information about their age, their body weight, etc) or due to technical reasons (not enough sample to run the assay). The serum of the remaining 712 participants was assayed for SARS-CoV-2 antibodies, DHEAS and vitamin D, 3 months post-vaccination. One hundred ninety-eight of them were also tested for SARS-CoV-2 antibodies 3 weeks after the second dose of the vaccine (figure 1A). The baseline characteristics (gender, age, BMI, smoking, diabetes, hypertension, use of statins) of our study population (n=712) can be seen in table 1.

SARS-CoV-2 antibodies decline from 3 weeks to 3 months post-vaccination
Paired serum samples from a part of cohort (n=198) were tested at 3 weeks as well as 3 months post-vaccination with

Figure 2
(A) SARS-CoV-2 antibody titre at 3 months post-vaccination plotted against age (n=712). Spearman’s r=−0.247, p<0.0001. Red dots indicate female gender and blue dots male gender. (B) Dehydroepiandrosterone sulfate (DHEAS) levels plotted against age at 3 months post-vaccination. Spearman’s r=−0.46, p<0.0001.
the second dose of BNT162b2 vaccine. The SARS-CoV-2 antibodies levels were assessed at both of these timepoints. Pairing was significantly effective (correlation coefficient \( r=0.8139, p<0.0001 \)) and paired t-test showed that the log antibody titre significantly declines with time (mean of differences between 3 months and 3 weeks is \(-0.23\pm0.34, p<0.0001\)) (figure 1B).

### Multiple linear regression analysis of the effect of various factors on SARS-CoV-2 antibody titre
To examine which factors can potentially affect the SARS-CoV-2 antibody titre in our cohort 3 months post-vaccination, we employed a multiple linear regression analysis model, including initially all considered parameters (gender, age, smoking, hypertension, hypertension, hypertension, diabetes, statin use, BMI, vitamin D and DHEAS levels). As BMI, DHEAS and vitamin D levels did not show a linear trend with the antibody titre, these variables were converted to categorical ones using quartiles for DHEAS and vitamin D, and the categories of underweight, normal weight, overweight and obese in the case of BMI. The results of the multiple linear regression analysis model that incorporates all these factors can be seen in table 2. Using stepwise entry criteria with \( p<0.05 \), the parameters diabetes, hypertension, prior statin use, BMI and DHEAS were excluded one by one with the aforementioned order rebuilding each time the linear regression model. Finally, the parameters that were found to affect significantly the antibody titre were age, gender, smoking and vitamin D levels (table 3).

### Influence of age and gender on SARS-CoV-2 antibody titre 3 months post-vaccination
Multivariate linear regression analysis highlighted a statistically significant decreased response of the antibody titre with increasing age (table 3, figure 2A). Male gender also appears to have a negative effect on the antibody titre (table 3). The interaction between age and gender was also found to be significant (\( p<0.0001 \), table 3), meaning that the effect of gender is modified by age and vice versa. As shown in figure 2A, the rate of decline in antibody titre with increasing age tends to be higher in men rather than in women.

Since DHEAS has been reported as an ageing marker with its concentration negatively correlated with age, we hypothesised that DHEAS levels might predict SARS-CoV-2

### Table 1 Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Whole study population</th>
<th>n=712</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>268 (37.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>444 (62.4%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.8±11.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7±4.9</td>
</tr>
<tr>
<td>Smokers</td>
<td>245 (34.4%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>50 (7%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>115 (16.2%)</td>
</tr>
<tr>
<td>Statin use</td>
<td>108 (15.2%)</td>
</tr>
</tbody>
</table>

Data show means±SD. BMI, body mass index.

### Table 2 Initial multivariable linear regression model for SARS-CoV-2 antibodies titre

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male)</td>
<td>-0.1041 (-0.1747 to -0.0335)</td>
<td>0.0039</td>
</tr>
<tr>
<td>Age</td>
<td>-0.01300 (-0.0164 to -0.0096)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smoking (yes)</td>
<td>-0.1006 (-0.1653 to -0.03599)</td>
<td>0.0023</td>
</tr>
<tr>
<td>Hypertension (yes)</td>
<td>-0.04540 (-0.1364 to 0.04560)</td>
<td>0.3276</td>
</tr>
<tr>
<td>Diabetes (yes)</td>
<td>-0.01871 (-0.1433 to 0.1059)</td>
<td>0.7681</td>
</tr>
<tr>
<td>Statin (yes)</td>
<td>0.06516 (-0.02489 to 0.1552)</td>
<td>0.1559</td>
</tr>
<tr>
<td>BMI (underweight)</td>
<td>-0.1507 (-0.4253 to 0.1239)</td>
<td>0.2816</td>
</tr>
<tr>
<td>BMI (overweight)</td>
<td>0.08446 (0.01126 to 0.1577)</td>
<td>0.0238</td>
</tr>
<tr>
<td>BMI (obese)</td>
<td>0.06556 (-0.01823 to 0.1494)</td>
<td>0.1249</td>
</tr>
<tr>
<td>DHEAS (Q3)</td>
<td>-0.03468 (-0.1218 to 0.05248)</td>
<td>0.4350</td>
</tr>
<tr>
<td>DHEAS (Q2)</td>
<td>-0.03305 (-0.1244 to 0.05827)</td>
<td>0.4776</td>
</tr>
<tr>
<td>DHEAS (Q1)</td>
<td>-0.1229 (-0.2226 to -0.02312)</td>
<td>0.0158</td>
</tr>
<tr>
<td>25(OH)D (Q3)</td>
<td>0.08445 (-0.001423 to 0.1703)</td>
<td>0.0539</td>
</tr>
<tr>
<td>25(OH)D (Q2)</td>
<td>0.1396 (0.05289 to 0.2262)</td>
<td>0.0016</td>
</tr>
<tr>
<td>25(OH)D (Q1)</td>
<td>0.1158 (0.02801 to 0.2035)</td>
<td>0.0098</td>
</tr>
</tbody>
</table>

The p values refer to this initial multivariable linear regression model that included all the examined variables in this study.

25(OH)D quartiles: Q4, 4.1–18.89; Q3, 18.9–25.67; Q2, 25.68–32.99; Q1, 33–69.8 ng/mL (\( p=0.0106 \)).

DHEAS quartiles: Q4, 0.3–89.59; Q3, 89.6–145.9; Q2, 146–217.39; Q1, 217.4–637.6 µg/dL (\( p=0.0866 \) underweight; BMI<18.5, normal weight; BMI=18.5–24.9, overweight; BMI=25–29.9, obesity; BMI>30 (\( p=0.0638 \)).

BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate.
antibody titres. Indeed, in our cohort we confirmed the known negative association between age and DHEAS level (Spearman’s r = −0.46, p < 0.0001, figure 2B) but multivariate linear regression analysis did not find DHEAS to be a significant predictor of antibody titre.

**Table 3** Final multivariable linear regression model for SARS-CoV-2 antibodies titre

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male)</td>
<td>−0.1100 (−0.1738 to −0.04617)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Age</td>
<td>−0.005231 (−0.009047 to −0.001416)</td>
<td>0.0073</td>
</tr>
<tr>
<td>Smoking (yes)</td>
<td>−0.1097 (−0.1737 to −0.04567)</td>
<td>0.0008</td>
</tr>
<tr>
<td>25(OH)D (Q3)</td>
<td>0.07601 (−0.009362 to 0.1614)</td>
<td>0.0809</td>
</tr>
<tr>
<td>25(OH)D (Q2)</td>
<td>0.1231 (0.03724 to 0.2091)</td>
<td>0.0050</td>
</tr>
<tr>
<td>25(OH)D (Q1)</td>
<td>0.08248 (−0.003949 to 0.1689)</td>
<td>0.0614</td>
</tr>
<tr>
<td>Gender (male):age</td>
<td>−0.01090 (−0.01631 to −0.005490) &lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Adjusted $R^2$=0.1355.

25(OH)D quartiles: Q4, 4.1–18.89; Q3, 18.9–25.67; Q2, 25.68–32.99; Q1, 33–69.8 ng/mL.

25(OH)D as categorical data Q1, Q2, Q3 compared with Q4 show p=0.0422.

The association of vitamin D and antibody titre was not linear and thus quartiles (Q4: 4.1–18.89, Q3: 18.9–25.67, Q2: 25.68–32.99, Q1: 33–69.8 ng/mL) of vitamin D levels were used instead in the multivariate linear regression model. Q1, Q2 and Q3 quartiles were compared with Q4. A statistically significant association was found between vitamin D levels and antibody response (p=0.0422, table 3). Specifically, individuals with vitamin D levels in the Q2 quartile showed a positive association with antibody titre (0.1231; 95% CI 0.03724 to 0.2091, p=0.0050), while Q1 and Q3 quartiles trended to have positive association (figure 5, table 3).

**DISCUSSION**

In the present work, the potential association of SARS-CoV-2 antibody titres after vaccination in volunteers consisting mainly of healthcare workers was tested with a variety of potential parameters. This study was designed to evaluate the effect of these potential parameters on the antibody titres per se and not on the rate of decline of antibodies over time. Age was found to be negatively associated with the antibody titres with men showing a trend for a steeper decline with increasing age compared with women (figure 2A). Ageing has long been considered a factor that affects immunity and has been recognised as an important factor in the current coronavirus pandemic. Aged individuals have shown higher mortality rates from COVID-19. The lower antibody titre of aged subjects after vaccination may suggest an impaired immune response and it can be a matter of consideration for providing the aged population with booster shots. Another interesting finding is that men trend to have a steeper decline of the antibody titre with increasing age (figure 2A). This finding is in accordance with another study that used a smaller number of subjects but assessed antibody levels in various timepoints from day 1 to day 50 post-vaccination. It was shown that octogenarians generate much less antibodies and that women older than 80 years old show higher titres. Other major studies detected a correlation of lower antibody concentrations after vaccination with male sex and older age. It is also noteworthy that age is negatively associated with antibody titre after SARS-CoV-2 infection. As men also appear to dominate in COVID-19 case fatality at least in some countries (reviewed in the literature), such findings warrant further investigation with focus on the mechanisms male gender and ageing can have such effects on immune response and COVID-19 outcomes.

In our cohort, the prevalence of smokers was relatively high, that is, 245/712 (34.4%). Smoking was also associated with a negative association between smoking and antibody titre (−0.01090; 95% CI −0.01631 to −0.005490, p < 0.0001, figure 2B). Multivariate linear regression analysis revealed that these variables do not affect antibody titre independently (table 2). Users of statin treatment were not found to have different antibody response compared with non-users (table 2).

**Smokers showed a blunted humoral response to vaccination**

In our cohort, 245 out of 712 subjects (34.4%) were smokers. Multivariate linear regression analysis revealed a negative association between smoking and antibody titre (−0.1097; 95% CI −0.1737 to −0.04567, p=0.0008, table 3). The mean antibody titre of smokers 988±781.4 vs 731.2±603.9 in non-smokers (figure 4B).

**Vitamin D status and humoral response to vaccination**

The association of vitamin D and antibody titre was not linear and thus quartiles (Q4: 4.1–18.89, Q3: 18.9–25.67, Q2: 25.68–32.99, Q1: 33–69.8 ng/mL) of vitamin D levels were used instead in the multivariate linear regression model. Q1, Q2 and Q3 quartiles were compared with Q4. A statistically significant association was found between vitamin D levels and antibody response (p=0.0422, table 3). Specifically, individuals with vitamin D levels in the Q2 quartile showed a positive association with antibody titre (0.1231; 95% CI 0.03724 to 0.2091, p=0.0050), while Q1 and Q3 quartiles trended to have positive association (figure 5, table 3).
concluded to be associated with lower antibody titre after vaccination (figure 4B, table 2). This finding is in agreement with a recent smaller Italian study28 and with a larger Japanese study25 examining the antibody response after COVID-19 vaccination. A meta-analysis of 19 clinical studies revealed that smokers have higher odds of COVID-19 disease progression compared with non-smokers29 indicating that smoking is associated with probably impaired immune response to SARS-CoV-2 or that comorbidities associated with smoking can lead to such a result. The effects of cigarette smoking to immunity has been studied through the years and is usually associated with immunosuppression.30 Hence, health policies aiming to reduce the smoking population should be more thoroughly implemented not only for the cancer prevention and cardiovascular benefits but also for the amelioration in immune responses.

Obesity has been officially recognised as a disease in some countries (for instance, by the American Medical Association) as it is a recognised risk factor for cardiovascular disease,31 cancer,32 diabetes, hypertension, dyslipidaemia and other comorbidities.33 During the current coronavirus pandemic, obesity has been recognised as a risk factor for greater COVID-19 severity.34 In our study, the simple index of BMI was calculated based on information provided by the participants. Analysis showed that BMI was not associated with the total antibody titre (figure 3A, table 2). This is in accordance with other studies.24 28 However, another index of central obesity, waist circumference, has been found to be inversely associated with
to be associated with reduced mortality. A meta-analysis also supported this notion but concluded that more and especially prospective studies are needed. With reference to the effect of statins on the humoral response, a report on influenza vaccination focusing mainly in old population showed that chronic statin use had an immunosuppressive effect. In our study, statins had no significant effect on the antibody levels post-vaccination (table 2) but it should be noted that only 15.2% of our cohort used statins (table 1).

Besides the anthropometric measurements and medical history based on the questionnaires, 25(OH)D and DHEAS levels were assessed in the subjects of our study. These hormones were selected for different reasons. DHEAS is an ageing marker and has been indirectly implicated in immune responses. Although we confirmed in our series the negative association of DHEAS levels with age (figure 2B), we could not find any association of DHEAS with the antibody levels (table 2). This means that the negative effect of age on antibody titres is not dependent on DHEAS levels and other mechanisms should be investigated.

Vitamin D has been in the spotlight of research for its pleiotropic actions besides bone effects for at least the last 2 decades. Its effects on immune response attracted our attention as they may affect the antibody titres after COVID-19 vaccination. Specifically, vitamin D supplementation has been shown to increase antibodies production after influenza vaccination in an elderly population, whereas vitamin D deficiency did not have any association with the immunogenic response to influenza vaccination according to a meta-analysis. Another study did not find any effect of vitamin D levels on post-vaccination antibodies against influenza. Herein, subjects with vitamin D levels in the range of 25.68–32.99 ng/mL had a positive association with higher antibody titres when compared with subjects in the lower range of vitamin D in our population. The other 2 quartiles of vitamin D 18.9–25.67 and 33–69.8 did not show a statistically significant association with higher antibody titres but only a trend as seen in figure 5. In our study, no information was available on whether our subjects were taking vitamin D supplementation and for how long. We just recorded vitamin D levels at a particular timepoint. Thus, it was not possible to know how long these levels were sustained and if there were previous fluctuations. The role of vitamin D in the antibody response after vaccination has not been systematically examined but future studies are warranted. The findings of the present study indicate that there may be a trend for increased antibody titres with higher vitamin D levels but given the fact that not all quartiles and especially the higher ones do not reach statistical significance indicates that further research is needed and possibly with a more thorough evaluation of the vitamin D levels over a long period of time and taking into account if the subjects are taking vitamin D supplements. It is worth mentioning that vitamin D supplementation has been tested even in
hospitalised patients with COVID-19 without definitive answers.\textsuperscript{46}

In conclusion, in the present study of 712 individuals, we show that the antibody titre generated after 3 months from the last dose of COVID-19 vaccination is negatively associated with age, smoking and male gender after the age of 40 years. No association was found between antibody titre and obesity, diabetes, hypertension or use of statins. DHEAS levels were negatively correlated with age but did not appear to have any effect on the antibody titre. Vitamin D levels showed a trend for a positive association with antibodies titre but not in all quartiles and thus further research is needed. Moreover, by measuring the antibody titre in 198 of our subjects 3 weeks post-vaccination we concluded that there is a decline in the titre between 3 weeks and 3 months. Limitations of our study include the reliance on questionnaire for anthropometric measurements and the reporting of diabetes and hypertension. Furthermore, no measures of neutralising antibodies or the cellular immune response have been performed in this study. However, the measurement of SARS-CoV-2 spike (S) protein RBD antibodies using the assay developed by Roche appears to be associated with the levels of neutralising antibodies in a growing number of studies.\textsuperscript{47–49} Another limitation of this study is that the final multiple linear regression model that was generated has an adjusted $r^2=0.14$ (table 3), meaning that this model explains roughly only 14% of the variance in our data and indicates that other parameters, besides the ones already taken into consideration, should be included so as to further improve the model. Moreover, as this study was carried out during a period that the delta variant of SARS-CoV-2 was the most prevalent and thus may have limited applicability in the current Omicron phase of the pandemic. Advantages of our study include the relatively high number of participants, the strict criterion of excluding all subjects that at least one parameter (of those examined) is missing, the use of a multiple linear regression model taking into account all parameters examined in parallel. Last but not least, to the best of our knowledge, this was the first study to examine DHEAS and 25(OH)D levels as potential modulators of the SARS-CoV-2 antibody titre response post-vaccination.

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**Contributors** AP, EEH, GIH, MM and DVC participated in designing this study. AP, EEH, GIH, AD and EL performed data collection. AP and DVC undertook the statistical analysis. AP, EEH, GIH, MM and DVC analysed and interpreted the data. AP and DVC wrote the first draft of the manuscript which was reviewed and approved by all authors. DVC is the guarantor of this work.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, conduct, or reporting or dissemination plans of this research.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by the University Hospital of Patras Ethics Committee (approval ID 99-25/2/2022). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request. Most data are available in the paper. Further datasets are available on reasonable request to the corresponding author.

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**REFERENCES**