# Coronavirus disease 2019 is associated with long-term depressive symptoms in Spanish older adults with overweight/obesity and metabolic syndrome

# Supplementary Methods

## PREDIMED-Plus Participants

In brief, 23 centres across Spain recruited participants from several primary healthcare facilities affiliated with the National Health System. Candidates were community-dwelling adults (men aged 55–75y; women aged 60–75y). Participants were eligible for study enrolment if they were overweight or obese (body mass index [BMI] between 27 and 40 kg/m2) and satisfied a minimum of three criteria for metabolic syndrome (Alberti et al., 2009). At enrolment, participants were free from CVD. Further inclusion and exclusion criteria are detailed in the protocol(Martínez-González et al., 2019) and accessible at <http://predimedplus.com/>.

## Ascertainment of Variables

#### Exposure: SARS-CoV-2 infection

A COVID-19 event (SARS-CoV-2 infection) was confirmed as per the following definitions: a) Confirmed COVID-19 was defined as a positive response to any one of the following tests: PCR SARS-CoV-2 (acute infection), Ag SARS-CoV-2 (acute infection), Ab SARS-CoV-2, total or IgG (past infection) and b) Probable COVID-19 infection was defined as Ab SARS-CoV-2, IgM + without a subsequent increase in IgG (IgG -) or a clinical presentation compatible diagnosis of COVID-19 by an attending physician treating COVID-19, without analytical testing at or after the acute moment. COVID-19 events adjudicated and confirmed until December 31st, 2021, were used in this analysis. Overall, 409 participants were confirmed COVID-19 cases and 1 participant was a probable case of COVID-19. Owing to only one of the COVID-19 positive cases designated as probable, both confirmed and probable cases of COVID-19 were pooled for this analysis as COVID-19 positive. Participants who did not have a confirmed or probable positive diagnosis of SARS-CoV-2 infection were considered COVID-19 negative, i.e., they were assumed to have not experienced the infection

#### Serology Analysis in a sub-sample for supplementary analysis

Participants were first tested for SARS-CoV-2 IgG using SARS-CoV-2 IgG ELISA Kit – (Enzo Biochem, New York, USA) (sensitivity: 100% and specificity: 96.5%) by duplicate. Participants who tested negative for the SARS-CoV-2 IgG were confirmed as COVID-19 negative. Participants whose serum tested positive in the above test were submitted to a second test using Technozym anti-SARS-CoV-2 RBD IgG test kit (Diapharma, West Chester, USA) (sensitivity: 99.3% and specificity: 100%). Those testing negative in the second test by duplicate were classified as COVID-19 negative and others were confirmed as COVID-19 positive.

## Assessment of confounder variables

PREDIMED-Plus has a scheduled collection of data that facilitates adjustments for potentially relevant confounders. Sociodemographic data (age, sex, educational level, marital status) and lifestyle information including physical activity (Molina et al., 2017), alcohol consumption and health status (e.g. presence or treatment for diabetes, hypercholesterolemia)were self-reported by the participants at face-to-face interviews at the baseline visit of the PREDIMED-Plus Trial and during the annual follow-ups. Anthropometric data were collected yearly, and BMI was calculated as weight in kg divided by the square of height in m2. Adherence to an energy-reduced Mediterranean diet was assessed during annual visits by using a validated 17-item energy-restricted Mediterranean Adherence Screener (er-MEDAS)(Schröder et al., 2021). Cognitive assessments were performed using a Spanish-validated version of the Mini-Mental State Examination (MMSE) questionnaire, commonly used as a cognitive screening test(Blesa, 2001; Folstein et al., 1975; Lobo et al., 2002). A higher MMSE score indicates better cognitive performance.

## Statistical Analysis

#### Preliminary cross-sectional exploration

A preliminary cross-sectional exploration of the data at the pre-COVID-19 visit was undertaken, descriptive statistics for continuous variables are shown as the median and interquartile range (IQR), and categorical data are displayed as count and percentages. To compare the pre-Covid-19 characteristics of COVID-19 negative and positive participants, Chi-square test and Mann-Whitney U test (owing to the skewed nature of the distribution) were used, as appropriate.

#### Models used for primary analysis

Model 1 adjusted for age, sex, education, marital status, intervention group, recruitment centre size, pre-COVID-19 BDI-II scores, and time since COVID-19 for depression assessments as confounders. Model 2 additionally adjusted for the presence of obesity (BMI >= 30 kg/m2), type 2 diabetes mellitus, hypertension, hypercholesterolemia, and cognitive performance on recruitment to the trial. Model 3, also adjusted for lifestyle factors including scores of adherence to the Mediterranean diet, total physical activity levels, smoking status and alcohol consumption (Table 2). Alcohol consumption was used as a quadratic term in the model to accommodate for a non-linear relationship with the outcome. All analyses were conducted with robust estimates of the variance to correct for intra-cluster correlation. This procedure was used to control for the allocation of household members into the same intervention group without randomization.

#### Secondary Analysis

A secondary analysis was performed with post-COVID-19 elevated depression risk as a binary outcome using BDI-II cut-offs of ≥ 14 (Table 3). Logistic regression was used to calculate Odds Ratios (ORs) and their 95% confidence intervals (CIs), considering the COVID-19 negative status as the reference category. The results were adjusted for the same confounding factors noted above, using the three same models developed for the main linear regression analysis.

#### Supplementary Analysis

#### Evaluation of minimal adjustment

In order to evaluate the effect of over-adjustments, a directed acyclic graph (DAG) was modelled (Supplementary Figure S2) based on existing literature (Textor et al., 2016). A minimal adjustment set to quantify the total direct effect of COVID-19 on depression measures included only pre-COVID-19 depression scores. Minimal linear and logistic regression models were accordingly tested as supplementary analysis (Supplementary analysis 1: Supplementary Table S1).

#### Lower BDI-II cut off to identify elevated depression risk

Evaluating the use of BDI-II in Spain over 50 years, Sanchez (2013) proposed that for the non-institutionalised Spanish population, a cut-off ≥ 12 had an adequate specificity index and diagnostic concordance and could detect major depressive episodes in 93% of individuals(Sanz Fernández, 2013). Thus, a supplementary analysis using a cut-off ≥ 12 was also conducted to categorise the outcome variable, namely the elevated risk of depression post-COVID-19. An additional supplementary logistic regression analysis was undertaken using a BDI-II score ≥ 12 as the cut-off for elevated depression risk (Supplementary analysis 2: Supplementary Table S1).

#### Evaluation of effect modification

Next, in order to assess the possible effect modification of association by potential confounders (age group [≤70 or <70 years], sex, intervention group, disease conditions and time elapsed post-COVID-19), product-terms were introduced in the different multivariable models. P-values for interaction were calculated with the likelihood ratio test and coefficient plots were generated to visualize the trends by strata (Supplementary Figure S2). Sub-analyses that stratified the results by factors that showed significant interaction (sex (Tables 2, 3)), presence of pre-COVID-19 high depression risk (Table 4) and time elapsed post-COVID-19 during depression assessments (Supplementary Table S2) were undertaken to identify strata specific trends. Post-estimation was performed for the final model to obtain predicted post-COVID-19 BDI-II scores. These were generated for the strata that showed significant interactions (Supplementary Figure S4). Finally, supplementary linear and logistic analyses were conducted in the sub-sample with serology results to ascertain COVID-19 status (Supplementary Table S3).

## References

Sanz Fernández, J. (2013). *50 years of the Beck Depression Inventory: Recommendations for using the Spanish adaptation of the BDI-II in clinical practice* [Info:eu-repo/semantics/article]. Consejo General de la Psicología de España. https://eprints.ucm.es/id/eprint/36450/

Textor, J., van der Zander, B., Gilthorpe, M. S., Liśkiewicz, M., & Ellison, G. T. (2016). Robust causal inference using directed acyclic graphs: The R package ‘dagitty.’ *International Journal of Epidemiology*, *45*(6), 1887–1894. https://doi.org/10.1093/ije/dyw341