**Online supplementary file**

**Physical activity and lung function - cause or consequence?**

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**Implementaton of SEMs**

SEMs were implemented using the gsem command of the STATA software, as follows:

*gsem (lf\_t2 <- lf\_t1 i.pa\_t2 [list V] [list L\_t1])*

*(pa\_t2 <- lf\_t1 [list V] [list L\_t1], logit)*

*(lf\_t3 <- lf\_t2 i.pa\_t3 [list V] [list L\_t2])*

*(pa\_t3 <- lf\_t2 [list V] [list L\_t2], logit)*

where:

- t1=ECRHS I, t2=ECRHS II and t3=ECRHS III

* pa\_t is physical activity (binary in our example) at time t
* lf\_t is lung function (i.e. the FEV1 or FVC measure in our example) at time t
* list\_V is the list of time\_fixed covariates: sex, education, age, age-squared, height, occupation, AHEI-2010 score, respiratory infection in childhood, and centre in our example (NB: the inclusion of occupational exposures compromised statistical power without substantially altering the results, thus it was not considered as covariate in the final models).
* list\_L\_t is the list of time-dependent covariates at time t: number of pack-years smoked, passive smoking exposure, weight in our example (NB: the inclusion of BMI (instead of weight) and menopausal status (in addition to age and age-squared) compromised statistical power without substantially altering the results, thus they were not considered as covariates in the final models).

The first line of the code models lung function at ECRHS II according to lung function at ECRHS I, physical activity at ECRHS II, time-fixed covariates and time-dependent covariates at ECRHS I, the second line models physical activity at ECRHS II according to lung function at ECRHS I, time-fixed covariates and time-dependent covariates at ECRHS I, the third line models lung function at ECRHS III according to lung function at ECRHS II, physical activity at ECRHS III, time-fixed covariates and time-dependent covariates at ECRHS II and the fourth line models physical activity at ECRHS III according to lung function at ECRHS II, time-fixed covariates and time-dependent covariates at ECRHS II.

**Implementation of MSMs**

Associations estimated in observational studies cannot usually be interpreted as causal effects, because the exposed and unexposed subjects are rarely exchangeable (i.e., exposed and unexposed subjects rarely share the same set of confounder values). MSMs address time-dependent confounding and, at the same time, allow estimation of causal effects in observational studies [1] by mimicking a hypothetical randomized experiment via creation of a pseudo-population in which exposed and non-exposed subjects are exchangeable within levels of the available confounders [2].

For that purpose, each subject will be assigned a weight proportional to the inverse of the probability that each subject had his own exposure (i.e. physical activity) history at a given time, given a chosen set of covariates [3]. Standardized weights for physical activity were stabilized and calculated as follows:

(a) *SW*(t) = ,

where SW(t) represents the stabilized weight for physical activity at time t, E1 represents physical activity, L represents a vector of time-dependent covariates, including previous lung function, and V represents a vector of time-fixed covariates. The probabilities in the numerator and denominator were estimated through logistic regression models for the probability of being physically active at each time s.

The second step consists of estimating the causal effect of physical activity on lung function in the reweighted pseudo-population using a weighted pooled linear regression model (i.e. mixed model) for lung function for each subject at each time t, according to physical activity at time t. As the set of baseline covariates V was not adjusted for through the weights, these variables were added as covariates in the final model [4].

MSMs were implemented using the STATA software, considering a pooled dataset in which one observation corresponds to one subject at each time-point (i.e. two observations per subject) as follows:

We define:

* id is the unique identifier for each subject
* time (i.e. 1 or 2) is considered as a continuous covariate in our example (i.e. we assume a linear association between time and exposure)
* pa\_t is physical activity (binary in our example) at time t
* pa\_lag is physical activity (binary in our example) at time t-1 (i.e. previous examination)
* lf\_t is lung function (i.e. the FEV1 or FVC measure in our example) at time t
* lf\_lag is lung function (i.e. the FEV1 or FVC measure in our example) at time t-1 (i.e. previous examination)
* list\_V is the list of time\_fixed covariates: sex, education, age, age-squared, height, occupation, AHEI-2010 score, respiratory infection in childhood, and centre in our example (NB: the inclusion of occupational exposures compromised statistical power without substantially altering the results, thus it was not considered as covariate in the final models).
* list\_L\_lag is the list of time\_dependent covariates at time t-1 (i.e. previous examination): lung function (i.e. lf\_lag), number of pack-years smoked, passive smoking exposure, weight in our example (NB: the inclusion of BMI (instead of weight) and menopausal status (in addition to age and age-squared) compromised statistical power without substantially altering the results, thus they were not considered as covariates in the final models).

1) First step: weight calculation according to the stabilized weight equation (a) described above:

*/\*\*\*\*\*\*\*\*\*\* Numerator \*\*\*\*\*\*\*\*\*\*\*/*

At each time t, according to the numerator of equation (a), we first model physical activity at time t according to physical activity at t-1 (only for t=2) and time-fixed covariates. Second, we estimate the probability of being physically active (using predict as a secondary step).

*\*For t=1 (ECRHS II)*

*xi:logistic pa\_t [list V] time*

*predict numpa if e(sample)*

*\*For t=2 (ECRHS III)*

*xi:logistic pa\_t i.pa\_lag [list V] time*

*predict numpa if e(sample)*

We calculate the numerator as the product of all time-specific probabilities of being physically active up to that time:

*sort centre id time*

*by centre id: replace numpa=numpa\*numpa[\_n-1] if \_n!=1*

*/\*\*\*\*\*\*\*\*\*\* Denominator \*\*\*\*\*\*\*\*\*\*\*/*

At each time t, according to the denominator of equation (a), we first model physical activity at time t according to physical activity at t-1 (only for t=ECRHS II), time-fixed covariates and time-dependent covariates at time t-1. Second, we estimate the probability of being physically active (using predict as a secondary step).

*\*For t=1 (ECRHS II)*

*xi:logistic pa\_t [list V] [list L\_lag] time*

*predict denpa if e(sample)*

*\*For t=2 (ECRHS III)*

*xi:logistic pa\_t i.pa\_lag [list V] [list L\_ lag] time*

*predict denpa if e(sample)*

We calculate the denominator as the product of all time-specific probabilities of being physically active up to that time:

*sort centre id time*

*by centre id: replace denpa=denpa\*denpa[\_n-1] if \_n!=1*

We calculate a weight for each subject at each time period, defined as explained in the equation above (a) by dividing the numerator by the denominator:

*gen w=numpa/denpa*

2) Second step: reweighted pooled model

We apply the calculated weights at each time and for each subject in the final pooled reweighted model (linear mixed model using the option [pw=w]) for lung function according to physical activity, adjusting for time-fixed covariates, allowing to assess the association between physical activity and lung function correcting for time-dependent confounding:

*xi: mixed lf\_t i.pa\_t [list V] time [pw=w] || id :*

**References**

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**Table S1.** Comparison between included and excluded subjects

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Included (n=753)** | **Excluded (n=825)** | **P** |
| **FEV1** (mL), m (SD) | 3.5 (0.7) | 3.5 (0.7) | 0.32 |
| **FVC** (mL), m (SD) | 4.3 (0.9) | 4.4 (1.0) | 0.78 |
| **Physical Activity** |  |  |  |
| **Active (%)** | 30.7 | 28.9 | 0.45 |
| **Number of pack-years smoked, m (SD)** | 21.5 (17.1) | 22.6 (17.2) | 0.22 |
| **Passive smoking (%)** | 65.2 | 69.5 | 0.07 |
| **Weight** (kg), m (SD) | 74.1 (14.7) | 73.4 (15.3) | 0.29 |
| **Menopausal status in women (%)**  Pre-menopausal  Post-menopausal | 84.2  15.8 | 85.7  14.3 | 0.69 |
| **Sex (%)**  Female  Male | 45.5  54.5 | 54.1  45.9 | 0.001 |
| **Education (%)**  <17 years  17–20 years  >20 years | 22.1  34.6  43.3 | 22.3  38.8  38.9 | 0.16 |
| **Age** (years), m (SD) | 41.4 (7.0) | 41.8 (6.9) | 0.22 |
| **Height** (cm), m (SD) | 170.2 (8.9) | 169.9 (9.4) | 0.60 |
| **Occupation (%)**  Management/professional/non-manual  Technical/professional/non-manual  Other non-manual  Skilled manual  Semiskilled/unskilled manual  Other/unknown | 26.6  18.9  23.9  13.6  13.0  4.1 | 26.3  17.1  25.2  10.9  10.6  9.9 | <0.001 |
| **Alternative healthy eating index-2010**, m (SD) | 50.2 (12.1)\* | 52.1 (12.5) | 0.03 |
| **Respiratory infection during childhood (%)** | 10.4 | 12.1 | 0.31 |
| **Occupational exposure to dust, gas/fumes or pesticides during follow-up (%)** | 53.4 | 50.4 | 0.24 |

m: mean; SD: standard deviation

**\*** The AHEI-2010 score was only available for 267 out of the 825 excluded subjects (as having available AHEI-2010 was one of the selection criteria)

**Figure S1.** Flow-chart of the study population

