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Sensitivity analysis for the effects of multiple unmeasured confounders

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Conflicts of Interest

All authors declared that they do not have any conflict of interest.

Abstract

Purpose: Observational studies are prone to (unmeasured) confounding. Sensitivity analysis of unmeasured confounding typically focus on a single unmeasured confounder. The purpose of this study was to assess the impact of multiple (possibly weak) unmeasured confounders.

Methods: Simulation studies were performed based on parameters estimated from the British Women's Heart and Health Study, including 28 measured confounders and assuming no effect of ascorbic acid intake on mortality. In addition, 25, 50, or 100 unmeasured confounders were simulated, with various mutual correlations and correlations with measured confounders.

Results: The correlated unmeasured confounders did not need to be strongly associated with exposure and outcome in order to substantially bias the exposure-outcome association at interest, provided that there are sufficiently many unmeasured confounders. Correlations between unmeasured confounders, in addition to the strength of their relationship with exposure and outcome, are key drivers of the magnitude of unmeasured confounding and should be considered in sensitivity analyses. However, if the unmeasured confounders are correlated with measured confounders, the bias yielded by unmeasured confounders is partly removed through adjustment for the measured confounders.

Conclusions: Discussions of the potential impact of unmeasured confounding in observational studies, and sensitivity analyses to examine this, should focus on the potential for the joint effect of multiple unmeasured confounders to bias results.

Key words: bias; confounding; sensitivity analysis

Introduction

Associations between exposures and health outcomes estimated in observational studies are prone to confounding. Observed confounders can be adjusted for in the analysis, but unmeasured and residual confounding can still bias estimated effects. For example, in a large cohort study ascorbic acid (vitamin C) was found to reduce all-cause mortality by 52% (RR 0.48, 95%CI 0.33-0.70, comparing highest with lowest quintile of ascorbic acid intake),[1] after adjustment for measured confounders (age, sex, body mass index (BMI), smoking status, systolic blood pressure, serum cholesterol, diabetes and vitamin C supplement use). Since this effect was not found in randomized trials (e.g. RR 1.00, 95%CI 0.94-1.06, for vitamin C supplementation vs. placebo),[2] it was disputed whether the observational study result was biased by unmeasured confounding by e.g. socioeconomic status or dietary habits.[3-5]

Publications describing methods for sensitivity analysis of unmeasured confounding typically focus on the impact of a single confounding variable.[6-15] It is often assumed that only a variable with a strong association with both exposure and outcome can materially confound the association under study. For example, in an observational study of influenza vaccine effectiveness, sensitivity analysis of unmeasured confounding was conducted by simulating a single unmeasured confounder under a wide range of scenarios. The authors concluded that "… our sensitivity analyses indicate how our estimates of vaccine effectiveness would be lower, though still significant, after adjustment for the effect of a strong hypothetical unmeasured confounder".[16] Similarly, a sensitivity analysis suggested that odds ratios between a confounder and both exposure and outcome would need to be at least 2.8 to nullify a positive association between use of a telephone health coaching service and hospital emergency admission rates. The authors concluded that unmeasured confounding was an

unlikely explanation because "… the amount of unobserved confounding would have had to be greater than is realistic for clinical variables".[17]

In the example of ascorbic acid intake and mortality, one can evaluate scenarios of a single binary unmeasured confounder that could cause the observed relation (OR 0.48) between ascorbic acid intake and mortality, if there were in truth no association. Several of such scenarios are shown in Figure 1 (based on the method described by Lin, Psaty, and Kronmal[8]). For example, an unmeasured binary confounder that is present in 25% of the population, increases the odds of the outcome seven times (OR 7), and is negatively associated with exposure (OR 0.2) would lead to an observed relation of OR 0.48, even though there were no association. Confounders that have such strong relations with exposure and outcome and are relatively common are probably known. Therefore, it may be hard to imagine that such a confounder exists, yet is unmeasured. This may therefore suggest that it is unlikely that the observed relation between ascorbic acid intake and mortality is due to unmeasured confounding.

<< FIGURE 1 >>

However, multiple unmeasured "weaker" confounders (i.e. each with a small association with both exposure and outcome) may, together, yield considerable confounding bias. These different representations of unmeasured confounding are displayed in Figure 2. The sufficient set of variables to control for confounding includes the set of measured confounders (Z) as well as the set of unmeasured confounders (U).[18] The set of unmeasured confounders could consist of multiple variables (e.g., $u_1 - u_4$). The possibility that there could be multiple (unmeasured) confounders for any given association has been confirmed by the finding that

many measured subject characteristics/confounders in cohort studies are associated with one another, much more than would be anticipated by chance.[19] Although multiple unmeasured confounders can be summarized into a single variable,[6,8] this may be hard to conceptualize and the literature provides little guidance on how to construct such a summary.

<< FIGURE 2 >>

We argue that focusing on a single unmeasured confounder may distract from the possibility that multiple (possibly weak) unmeasured confounders can have a large joint effect. Therefore, methods or sensitivity analyses to quantify the impact of unmeasured confounding should also consider scenarios of multiple unmeasured confounders. Here, guidance is provided and sensitivity analysis of multiple unmeasured confounders is illustrated by an example of ascorbic acid intake and mortality.

The bias due to multiple confounders

The magnitude of the bias due to a confounder of the exposure-outcome association depends on: 1.) the strength of the association between confounder and exposure; 2.) the strength of the association between confounder and outcome; and 3.) the prevalence of the confounder in the population (for categorical confounders) or its variance (for continuous normally distributed confounders).[8] The direction of the bias depends on the signs of the association between the confounder and the exposure and outcome.[20] As the variance of a continuous confounder increases, so does the magnitude of the bias due to confounding.

The joint confounding effect of two continuous confounders depends on: 1.) the strength of the associations with exposure for each of the confounders; 2.) the strength of the associations with the outcome for each of the confounders; 3.) the variance of each of the confounders; and 4.) the covariance between the confounders. This follows from the fact that the variance of the combination of two continuous confounders is given by:

$$var_{A+B} = var_A + var_B + 2cov_{AB}$$

where var_A and var_B indicate the variance of the continuous confounders A and B, respectively, and cov_{AB} the covariance between these confounders. Similarly, the variance of the combination of more than two confounders can by calculated taking each pairwise covariance into account. Because the confounding effect of a continuous confounder depends on the variance of that confounder, the joint effect of multiple confounders depends on the variance of each of the confounders as well as their covariance. For example, the total confounding effect by two confounders that are positively correlated will be larger than the confounding effect by the two confounders if they were independent. Conversely, if two confounder are inversely correlated (i.e., negative covariance) the biases they introduce may (partly) cancel out.

When there are several confounders, all with the same direction of effect with exposure, and all with the same direction of effect on the outcome, the effect estimate that is adjusted for a subset of these confounders ('partially adjusted') will be closer to the true exposure-outcome association than the unadjusted estimate.[21] However, if the effects of some of the confounders on either exposure or outcome are in different directions, the partially adjusted estimate can actually be further away from the true exposure-outcome association than the unadjusted

estimate, particularly if the confounders are weakly correlated with one another. When the confounders are highly correlated, there is little confounding bias remaining after adjustment for a subset of the confounders, irrespective of the direction of confounding.[21] This is illustrated in Figure 3.

<< FIGURE 3 >>

If all confounders are considered unmeasured, the amount of bias is inversely proportional to the variance of the exposure variable. Hence, as the variance in the exposure increases (or the proportion of variance explained by confounders, R2, decreases), the bias due to unmeasured confounding will decrease. If a subset of the confounders is considered unmeasured, obviously only the measured confounders can be adjusted for in the analysis. In that case, the bias is inversely proportional to the variance of the exposure variable and proportional to the variance of the combination of the unmeasured confounders, which decreases as the number of confounders that is adjusted for increases. The actual shape of the curve of the relation between the number of confounders that is adjusted for and the residual bias is a multipart function of the number of confounders that is adjusted for, the mutual correlation between the confounders, and the variance of the exposure variable (details in Appendix A).

Informally, one could say that after adjustment for several correlated confounders, adding more confounders to the adjustment model will not make much difference, since the information captured by these new confounders is already part of the confounders included in the adjustment model. After including a number of confounders in the adjustment model, the exposure-outcome association indeed often appears to stabilize (little change in the estimate of the exposure-outcome association is observed when additional confounders are added).[22,23]

This implies that all (important) confounders are included in the model, if confounders are highly correlated. This relative stabilization of the exposure-outcome association upon adjustment for an increasing number of confounders clearly depends on the joint association between confounders. Note that if the unmeasured confounders are independent of the confounders that are included in the adjustment model, the observed stabilization falsely suggests absence of unmeasured confounding.

Sensitivity analysis of multiple unmeasured confounders

The Text box provides a description of the different steps that are needed when performing a sensitivity analysis of unmeasured confounding, when this unmeasured confounding may be the result of multiple unmeasured confounders. We stress that, as with any sensitivity analysis, it is necessary to make assumptions about the unmeasured confounders. The value of the analysis is therefore dependent on the quality of those assumptions. Even when supported by e.g., scientific literature these remain assumptions.

This approach is illustrated by the example of ascorbic acid intake and the risk of mortality. The number of unmeasured confounders, as well as their characteristics are varied. However, we assumed all unmeasured confounders to be normally distributed variables, which is an approach others have taken before.[8]

Text box. Step by step guidance on sensitivity analysis of multiple unmeasured confounders.

0. Based on the empirical data:
    a. Derive the distributions of measured confounders, exposure, and outcome
    b. Obtain estimates of the relations (e.g. covariance) between confounders
    c. Obtain estimates of the relations between confounders and exposure
    d. Obtain estimates of the relations between confounders and outcome
1. Make assumptions about the unmeasured standardised confounders, in particular specify:
    a. The number of unmeasured confounders
    b. The correlations between confounders and exposure
    c. The correlations between confounders and outcome
    d. The correlations between unmeasured confounders
    e. The correlations between unmeasured confounders and measured confounders
2. Simulate unmeasured confounders based on the assumptions made in step 1
3. Evaluate the exposure-outcome relation, accounting for the (simulated) unmeasured as well as the measured confounders
4. Repeat steps 1-3 for various scenarios
5. Discuss the plausibility of those scenarios for which the exposure-outcome association estimated in step 3 is consistent with the null, to answer the question "If in truth the exposure does not cause the outcome, how much unmeasured confounding needs to be present to find the observed exposure-outcome relation?"

Example: ascorbic acid intake and risk of mortality

We used simulated data to illustrate the bias caused by the joint effect of multiple unmeasured confounders. The basis for these data was the example of the study of the association between ascorbic acid intake and all-cause mortality. The parameters of the simulation are based on empirical data from the British Women's Heart and Health Study (BWHHS), see Appendix B for details.[3,5,24] These data were used to inform this simulation study on the impact of unmeasured confounding and not to draw causal conclusions. To provide realistic distributional assumptions for our simulation, we used baseline information on these women measured at inclusion in the cohort (1998 – 2000), together with follow-up data on mortality until September 30, 2007. For our simulations we used information on 28 confounding variables (based on the list in [4] and known risk factors for mortality), including demographic characteristics, socioeconomic indicators, behavioural and lifestyle risk factors, biomarkers, co-morbidity status, and medication use. The distribution of confounding variables among ascorbic acid groups is presented in Table A1 in Appendix B. The (Pearson) correlations between the confounding variables that are listed in Table A1 ranged between -0.37 and 0.46 (95% of the observed correlations between -0.17 and 0.28). Associations between the confounding variables and ascorbic acid and mortality are presented in Table A2 in Appendix B.

Simulation study

For all simulations and analyses we used R for windows, version 3.0.3.[25] We simulated datasets of 200,000 subjects in order to reduce simulation error. First, the 28 confounders were drawn from the multivariate normal distribution based on the BWHHS-based distributions given in Table A1 (Appendix B), using the function `mvrnorm()` from the library `MASS`. Six out of 28 confounders were continuous variables (age, body mass index (BMI),

leg-trunk ratio, systolic blood pressure, serum HDL cholesterol level, and serum LDL cholesterol level) and these were transformed into z-scores and assumed to be normally distributed. To create the 22 dichotomous confounders, continuous variables were created by again sampling from the multivariate normal distribution. Then, these continuous variables were dichotomized at zero. By fixing the mean of the continuous variable, the proportion of subjects in whom the dichotomous confounder is present can be controlled, while approximating the predefined (tetrachoric) correlation with other variables.

To assess the impact of unmeasured confounding, we generated 25, 50, or 100 "unmeasured confounders" in addition to the 28 confounders that were observed in the BWHHS cohort. They were standard normally distributed variables and also sampled from a multivariate normal distribution. The Pearson correlation between the unmeasured confounders was varied between 0 and approximately 0.15. The unmeasured confounders were either completely independent of the measured confounders, or the correlation between the unmeasured and the 28 measured confounders was the same as the correlation between the unmeasured confounders (e.g., if the correlation between unmeasured confounders was set at, say, 0.05, the correlations between the measured and unmeasured confounders was also set at the value 0.05).

After generating the measured and unmeasured confounders, the binary exposure was simulated by the following logistic model, which includes *n* confounders (28 measured confounders + 25, 50, or 100 unmeasured confounders):

$$logit(p_{i,x}) = a_0 + a_1 confounder_1 + a_2 confounder_2 + \ldots + a_n confounder_n$$

The exposure was simulated by sampling from a Bernoulli distribution with $p_{i,x}$ the probability of success. For the measured confounders in this model, the coefficients of the model were based on the conditional associations between the confounders and ascorbic acid status observed in the BWHHS data (listed in Table A1 in Appendix B). For the unmeasured confounders in the model, the coefficient was varied between –log(1.01) and –log(1.25), in order to induce a negative confounding effect. Because our aim was to assess the maximum impact of multiple unmeasured confounders, we only considered scenarios in which the effects of the unmeasured confounders were in the same direction: if the effects of the unmeasured confounders were in opposite directions their joint confounding effect would be smaller. The coefficient a_0 was set at 0.87 to arrive at a prevalence of exposure (highest vs. lowest quartile of ascorbic acid intake) of approximately 50%.

Next, the binary outcome was simulated by the following logistic model, which includes the exposure and the same *n* confounders as in the exposure model:

$$logit(p_{i,y}) = b_0 + b_x exposure + b_1 confounder_1 + b_2 confounder_2 + ... + b_n confounder_n$$

The outcome was simulated by sampling from a Bernoulli distribution with $p_{i,y}$ the probability of success. For the measured confounders, the coefficients of the model were based on the conditional associations between the confounders and the mortality observed in the subsample of the BWHHS data (Table A2 in Appendix B). For the unmeasured confounders in the model, the coefficient was varied between log(1.01) and log(1.25). The effects of the unmeasured confounders were in the same directions for the reasons described above. The exposure effect (b_x) was set at 0 (i.e., OR=1.00), indicating no association between ascorbic acid intake and mortality risk. The coefficient b_0 was set at -2.37, such that an event occurred

in approximately 20% of the simulated subjects, which is in line with the mortality rate observed in the subsample of the BWHHS cohort.

Analysis

After simulating the datasets, the impact of unmeasured confounding was evaluated. In each simulated dataset, the effect of ascorbic acid on mortality was estimated by logistic regression analysis. The 28 confounders listed in Table A1 in Appendix B were considered measured and adjusted for by including them as covariates in the logistic regression model. The other simulated confounders were considered unmeasured and hence omitted from the adjustment model. In line with the motivating example, we simulated scenarios where the true odds ratio for the ascorbic acid-mortality association was 1.0, but that yielded an odds ratio of the ascorbic acid–mortality association of approximately 0.48 after adjustment for the 28 measured confounders. An annotated excerpt of the simulation code is provided in Appendix C.

Results

Figure 4 shows the impact on the relation between ascorbic acid intake and mortality of consecutively adding confounders to the adjustment model. For this figure, a scenario was considered in which in addition to 28 measured confounders there were 25 additional unmeasured confounders. When all additional confounders are considered unmeasured (left side of graph), the bias of the exposure-outcome association depends on the correlation between the confounders (as explained above). This bias decreases as the number of confounders that is adjusted for increases.

<< FIGURE 4 >>

In Figure 5, the results are presented for scenarios in which all the unmeasured confounders have the same mutual correlation, yet they are independent of the measured confounders. Hence, there is no indirect adjustment for the unmeasured confounders by the measured confounders. In this scenario, the estimated OR decreases with increasing correlation between the unmeasured confounders. Correlated unmeasured confounders do not need to be strongly associated with exposure and outcome in order to bias the exposure/outcome association considerably, provided that there are sufficiently many of them.

<< FIGURE 5 >>

In the scenarios presented in Figure 6, the unmeasured confounders have the same mutual correlation, which is equal to their correlation with the measured confounders. As the number of unmeasured confounders increases, the association with both exposure and outcome that is required in order to observe the biased association (OR=0.48) between ascorbic acid intake and mortality becomes smaller. For correlations between the confounders of 0.05 and higher, the required association of the unmeasured confounders with exposure and outcome stabilized (ORs of approximately 1.18, 1.10, and 1.05 for the association of the unmeasured confounders with both exposure and outcome in case of 25, 50, and 100 unmeasured confounders, respectively). This stabilization is due to a trade-off between increased bias caused by increasing correlations between the unmeasured confounders and decreased bias caused by indirect adjustment for the unmeasured confounders by the measured confounders.

<< FIGURE 6 >>

Discussion

Our study, based on empirical data, shows that the bias caused by multiple unmeasured confounders can be substantial, even if these unmeasured confounders are themselves only weakly associated with exposure and outcome. This especially holds true if the unmeasured confounders are mutually correlated and independent of the measured confounders (the latter are adjusted for in the analysis). However, if the unmeasured confounders are also correlated with measured confounders and the correlations are all in the same directions, the bias by unmeasured confounders is partly reduced through adjustment for the measured confounders.

Various sensitivity analyses have been proposed.[6-15] Some focus on unmeasured confounding only,[7-11,13-15] whereas in other publications an encompassing framework for unmeasured and poorly measured confounding, together with measurement error and other sources of bias sensitivity analyses is proposed.[6,12] Often sensitivity analyses of unmeasured confounding focus on a single unmeasured variable.[7-10] Consequently, when thinking about the potential for unmeasured confounding in observational studies, researchers will often have a single unmeasured variable in mind. If sensitivity analysis then indicates that the unmeasured variable needs to have associations with exposure and outcome so strongly that it is deemed unrealistic that such a confounder exists, it is argued that the potential for unmeasured confounding is probably small.[17] Our study shows that this approach can be inappropriate and implies that discussions of the potential impact of unmeasured confounding in observational studies, and sensitivity analyses to examine this, should focus much more on the potential for the joint effect of multiple unmeasured confounders.

The potential impact of unmeasured confounding has also been assessed by others, using either empirical or simulated data.[7-11] Our simulations were based on an empirical dataset

from the BWHHS cohort. This can be considered a limitation, since it hampers generalizability of our findings. On the other hand it shows how such simulations can be used as a sensitivity analysis of unmeasured confounding within a real applied study where there is concern about unmeasured confounding. With a realistic set of assumptions about the number and distribution of unmeasured confounders, we were able to replicate the OR that was observed in the motivating example (OR 0.48). Thus, the observed association between circulating ascorbic acid and all-cause mortality in the BWHHS (and similar results in other cohort studies) could be plausibly due to unmeasured confounding. A discussion about which participant characteristics could be the reason for this confounding effect or about other possible reasons for the observed association (e.g. measurement error) is beyond the scope of this manuscript.

The current study shows how sensitivity analysis of multiple unmeasured confounders can be implemented using empirical data. We stress that in our simulations the confounding effects of all unmeasured confounders have the same direction, which allowed us to assess the maximum impact of these unmeasured confounders. This worst-case scenario is relevant for sensitivity analysis, even if it may not reflect reality completely.

Importantly, the impact of unmeasured confounders depends on their correlation with the measured confounders that are already adjusted for in the analysis. In observational studies, confounders are almost always mutually related.[19] We therefore consider the simulated scenario in which the unmeasured confounders are related to the measured confounders more realistic than the scenarios in which the two sets of confounders were assumed to be independent.

A limitation of our approach is that we focused on binary outcomes that are analyzed using logistic regression, while in empirical research often time-to-event data are analyzed. For a single unmeasured confounder, the method for sensitivity analysis proposed by Lin, Psaty and Kronmal has the same form for logistic models and survival models.[8] In both cases, the observed association between exposure and outcome is multiplied by a factor that represents unmeasured confounding. Our approach can be likewise extended to time-to-event data, in which case time-to-event (or censoring), as well as the outcome status would need to be simulated.[26]

In our simulations, we evaluated unmeasured confounding as a possible reason for the discrepancy between randomized and nonrandomized studies regarding the effect of ascorbic acid on mortality. Other reasons for this discrepancy include (differential) misclassification of the exposure or confounders, selective loss to follow-up, missing data and different durations of ascorbic acid exposure. Randomized controlled trials assess incident administration of a treatment, whereas cohort studies rarely examine exposure in that way.[27] In our example, blood measures of ascorbic acid should be a reasonable proxy for incident (current/baseline) intake of ascorbic acid.

We conclude that discussions of the potential impact of unmeasured confounding in observational studies – either etiological or non-randomized intervention studies – should focus much more on the joint effect of multiple unmeasured confounders. In addition to the strength of the associations of the unmeasured confounders with exposure and outcome, their mutual correlation is a key driver of the magnitude of bias resulting from unmeasured confounders. We support the recommendation by others,[6,8-12] that researchers of observational studies should routinely perform sensitivity analysis of (multiple) unmeasured confounders in

order to guide the discussion on the possible direction, magnitude and impact of these. Expert knowledge of the particular research question should be used to guide values for the plausible number, correlation and associations of potential unmeasured confounders to be used in such sensitivity analyses.

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FIGURES

Figure 1. Scenarios of unmeasured confounding of the relation between ascorbic acid intake and mortality that nullify the observed relation (OR 0.48).

Legend Figure 1:

Based on the method described by Lin, Psaty, and Kronmal.[10] The dotted line indicates a scenario in which an unmeasured confounder increases the odds of the outcome seven times, is negatively associated with exposure (OR 0.2), and is present in 25% of the population.

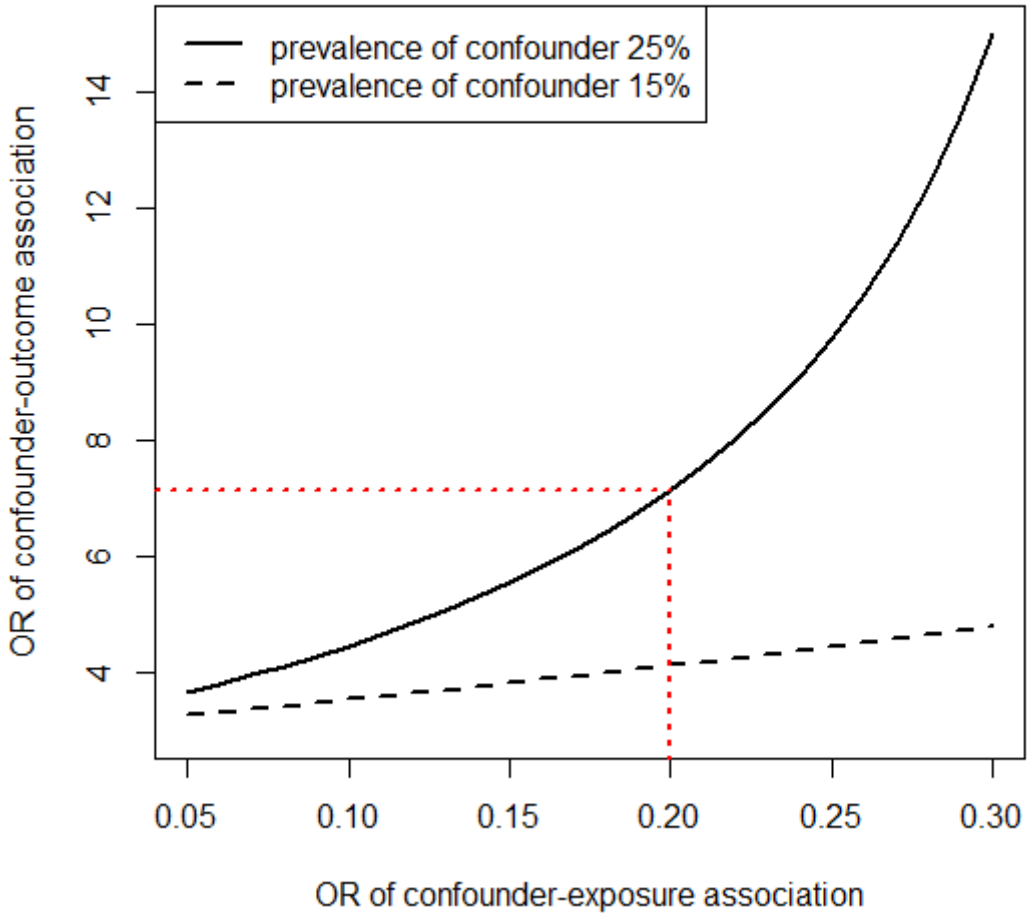


Figure 2. Causal diagrams of unmeasured confounding.

Legend figure 2:

X represents exposure, Y outcome, Z the set of measured confounders, and U represents the set of unmeasured confounders (which may consist of multiple 'weaker' confounders, u_1–u_4).

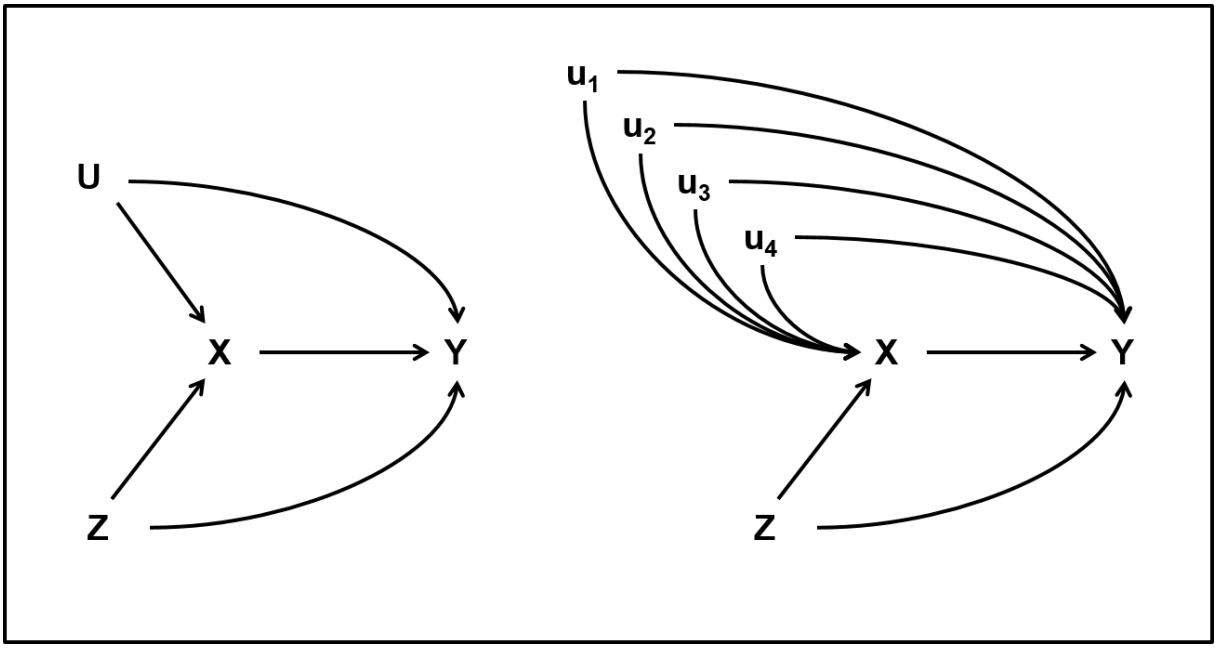
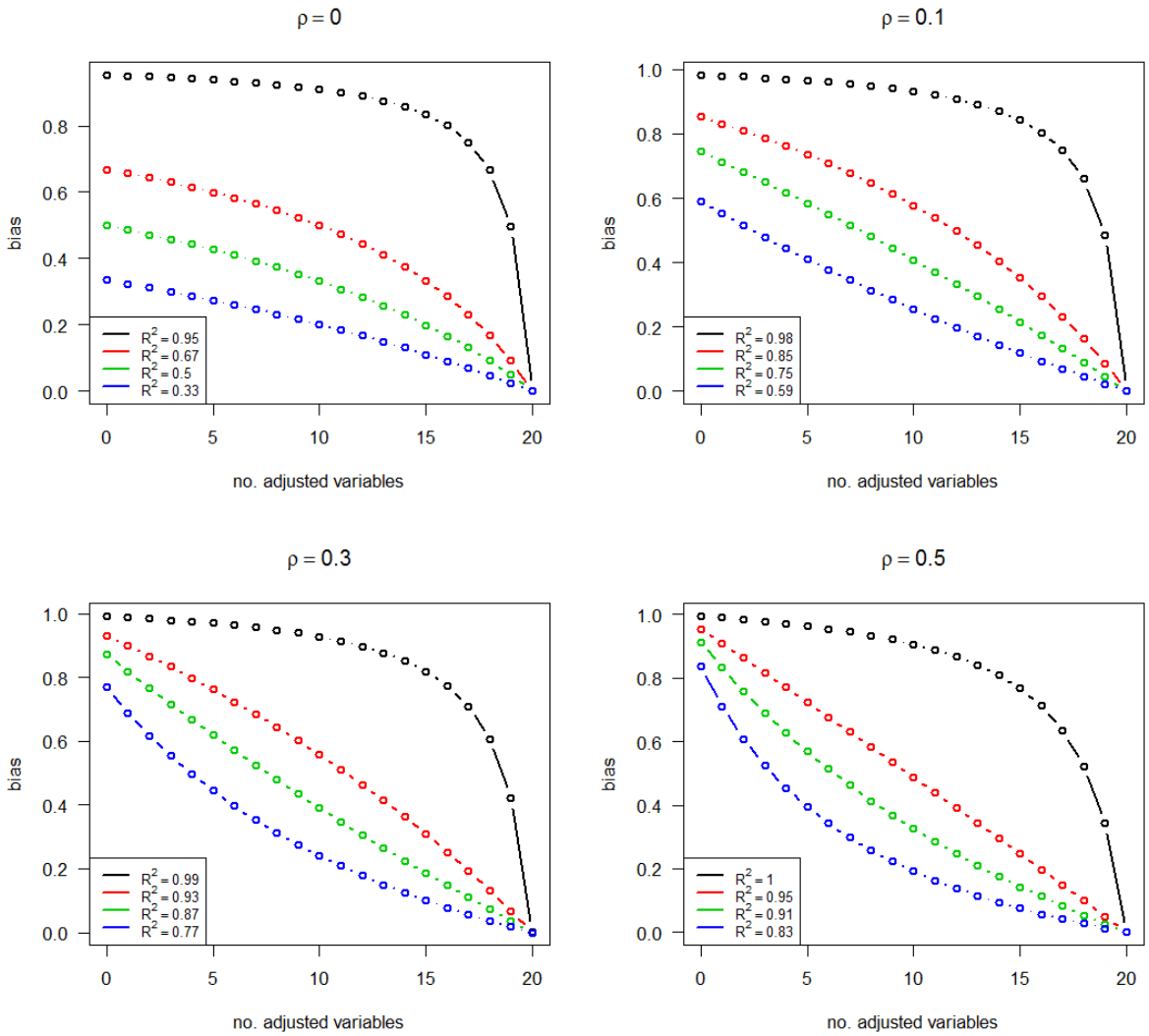


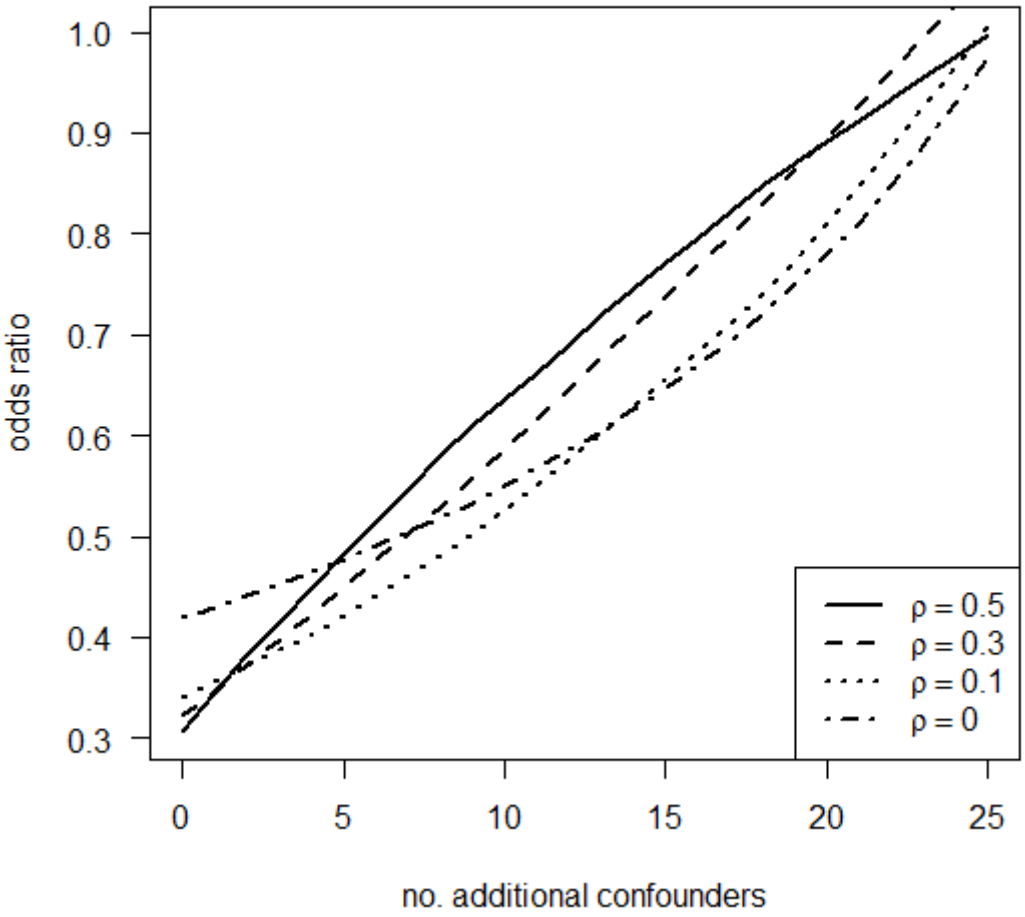
Figure 3. Relation between adjustment for a subset of the confounding variables and bias of the exposure-outcome relation in simulated data.



Legend figure 3:

Graphs are based on simulated data of 20 standard normally distributed confounders (with mutual correlation ρ). For each of the confounders, the values of exposure and outcome (both continuous, normally distributed) increase by one unit for every unit increase in any of the confounders. The explained variance (R^2) indicates the variance in the exposure that is explained by the 20 confounders; i.e., in scenarios with smaller R^2 values, more random error is added to the exposure variable, compared to scenarios with larger R^2 values.

Figure 4. Impact of consecutively adding confounders to the adjustment model on the estimated relation between ascorbic acid intake and mortality.

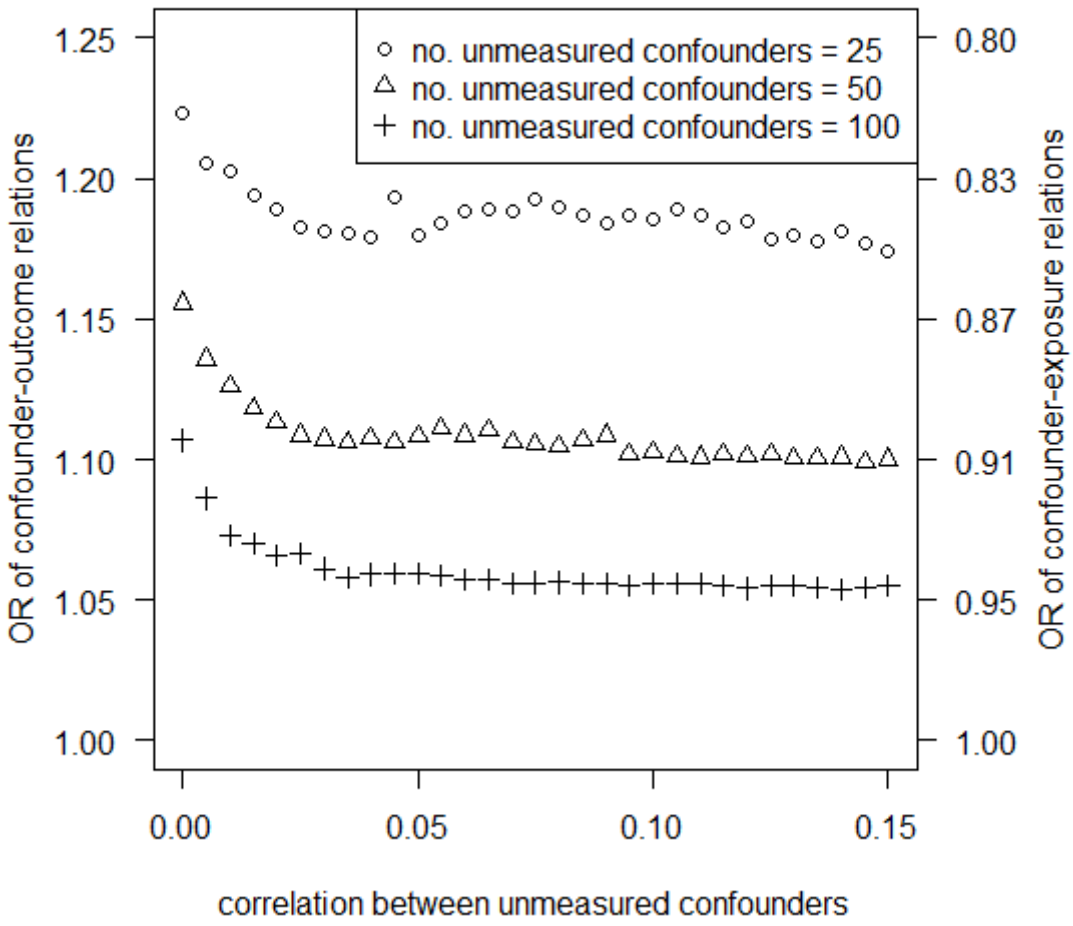


Legend

figure 4:

Results based on simulations. In all scenarios the true association between ascorbic acid intake and mortality risk was OR=1. In addition to 28 confounders that were considered measured, there were 25 additional "unmeasured confounders", which were all considered unmeasured (no. additional confounders = 0) or all considered measured (no. additional confounders = 25). The measured and unmeasured confounders were mutually correlated, indicated by Pearson's correlation (ρ).

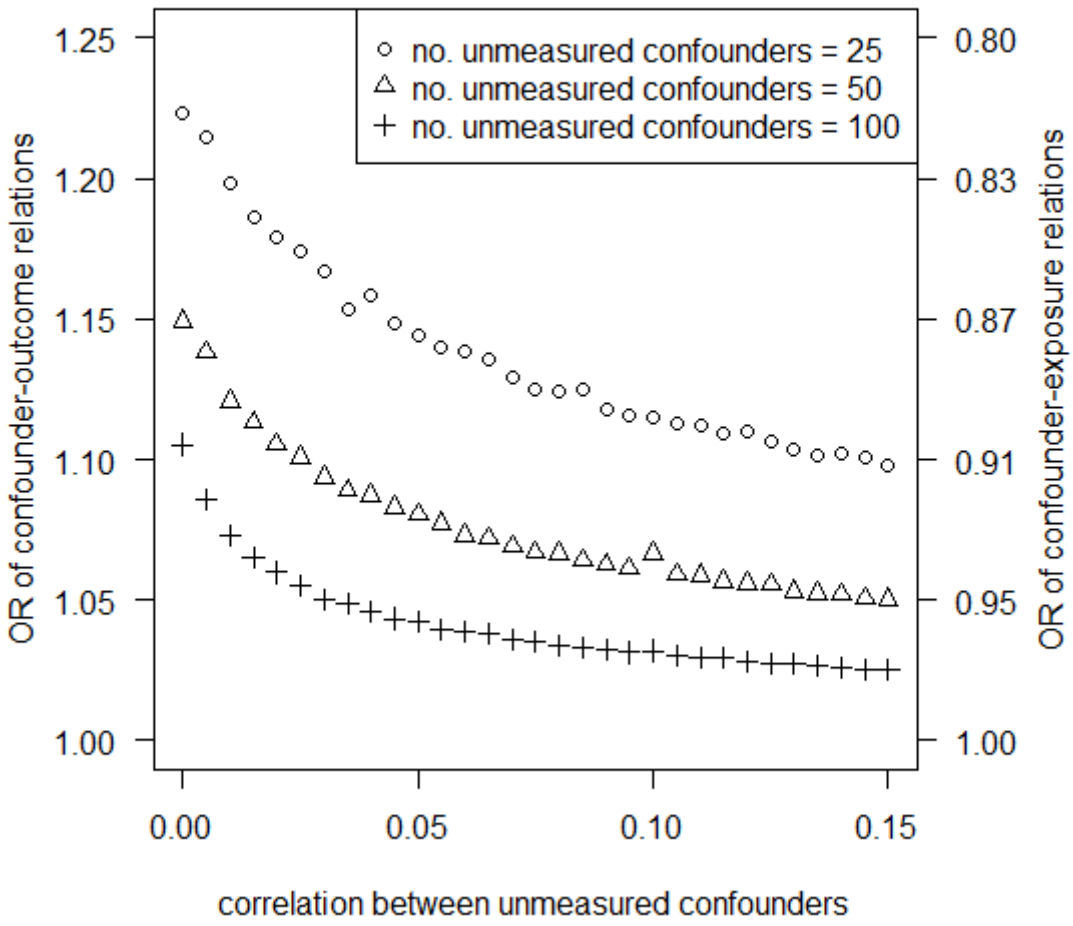
Figure 5. Overview of scenarios that resulted in a biased association (odds ratio 0.48) between ascorbic acid intake and mortality risk.



Legend figure 5:

In all scenarios the true association between ascorbic acid intake and mortality risk was OR=1. The unmeasured confounders were mutually correlated, but independent of the measured confounders.

Figure 6. Overview of scenarios that resulted in a biased association (odds ratio 0.48) between ascorbic acid intake and mortality risk.



Legend figure 6:

In all scenarios the true association between ascorbic acid intake and mortality risk was OR=1 and the unmeasured confounders were correlated with the measured confounders.