Methods for using published data to determine causal associations: assessing the association between obesity and asthma

Authors:
Grace Carmichael
Jared Tavares

Student Numbers:
CRMGRA004
TVRJAR001

Supervisors:
Professor Francesca Little
Ms Noëlle van Biljon

Department:
Statistical Sciences

October 24, 2022

1 Acknowledgements

We would like the thank our supervisors, Professor Francesca Little and Noëlle van Biljon, who guided us through this project and ensured that we always stayed on track. We would specifically like to thank Francesca for always providing clear and helpful feedback and Noëlle for the genetic expertise and helpful resources. Without their assistance, this project would not have been possible.

2 Abstract

To explore possible associations and identify causality using summary data; a systematic review, meta-analysis and Mendelian randomisation were conducted for the association between obesity and asthma. Conventionally, assessing causal relationships requires a randomised controlled trial (RCT). However, there are situations in which these trials are unethical or impractical to implement. Mendelian randomisation is a method for determining causality between two variables without needing RCTs. It involves using genetic variants (Single Nucleotide Polymorphisms, or SNPs) as proxies for the exposure of interest and assessing the relationship between these SNPs and the outcome of interest. This ability to determine causality in the relationship between two variables is necessary to implement correct treatment and prevention, especially in the health sciences.

For the meta-analysis, a three-level mixed effect model and a Bayesian hierarchical model were fitted to estimate the relationship between obesity and asthma. The meta-analysis was followed by a Mendelian randomisation where an inverse variance weighted model and a Mendelian randomisation-egger (MR-Egger) regression model were fitted to summarised genetic data obtained from Genome-Wide Association Studies (GWAS). Both of these models were fitted using a frequentist and Bayesian approach. The results from the Mendelian randomisation were similar to the results from the meta-analysis and both indicated that there is a possible association between obesity and asthma; however, this was not highly significant. The Bayesian and frequentist approaches for both methods produced similar estimates of the association between obesity and asthma. These methods have all been implemented in this context in the past but they have not all been combined to produce a holistic overview of the association between an exposure and outcome.

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3 Introduction

The use of summary data, especially in the health sciences, can be useful for assessing the association between two traits of interest. When using the results from multiple studies to assess an association, it can strengthen the results and conclusions made from the research. This is because multiple studies confirming the same results allows for more confidence in those results than a single study does. Summary statistics are also easily accessible and their use removes the need for expensive and complicated data collection methods.

Assessing causality in a relationship can be challenging, especially when randomised controlled trials are not possible. This review aims to demonstrate the use of summary data to assess an association between an exposure and outcome and then determine the direction of causality in this association.

3.1 Description of the exposure

Obesity is a disorder characterised by excess fat accumulation in the body and occurs as a result of a complex interplay between genetics, dietary caloric intake and energy expenditure (Adeniyi and Young, 2012). It is defined using the body mass index (BMI, measured as weight in kilograms/square of height in metres - (kg/m^2)). An adult individual with a BMI $\geq$ 30 would be classified as obese and this definition is accepted internationally (James, 2004). Obesity is associated with a high risk of many chronic diseases and therefore constitutes a major health problem (Obesity: preventing and managing the global epidemic, 2000). Subsequently, the dramatic increase in the prevalence of obesity worldwide has lead the World Health Organisation (WHO) to declare it a global pandemic (WHO, 2000).

3.2 Description of the outcome

Asthma is a disease in which there are episodes of reversible airway obstruction caused by chronic airway inflammation as well as airway hyper-responsiveness (Global strategy for asthma management and prevention, 2010). Asthma, like obesity, is also a serious public health concern with an increasing prevalence globally. With an estimated 300 million people of varying ages and ethnic backgrounds affected worldwide, asthma accounts for about one out of every 250 deaths (Masoli, Fabian, Holt and Beasley, 2004). Identifying factors that cause this condition is crucial for improved prevention and treatment.

3.3 Obesity and Asthma

The dramatic increase in the prevalence of both obesity and asthma over the last few decades has spurred an interest in the possible association between these conditions (Masoli, Fabian, Holt and Beasley, 2004).

A 1986 cross-sectional study was the first of its type and suggested a higher prevalence of asthma among obese Dutch men compared to those with normal weights (Seidell et al., 1986). Another cross-sectional study in 1998 showed that among American children, there was an excess of obesity in patients with asthma compared to those without asthma (Luder, Melnik and DiMaio, 1998). The first prospective study was conducted in 1999, which found a positive association between obesity and asthma. This provided different insight into the association than the cross-sectional studies that came before (Camargo et al., 1999). Since then, there have been many studies published assessing this association, including a meta-analysis of seven prospective studies.This showed an increase in the odds of incidence of asthma among the overweight (BMI $\geq$ 25) and the obese (BMI $\geq$ 30). This meta-analysis also found no difference in the association between sexes (Beuther and Sutherland, 2007). In addition to this, obesity is also associated with poorer management of asthma symptoms, in general (Lavoie et al., 2006). The management of symptoms is not really of interest here, but will be explored in the systematic review for a broader understanding of the association between obesity and asthma.

A study by Xu et al. (2019) investigated the relationship between obesity and asthma using a Mendelian randomisation approach. This study found that obesity has a positive causal effect on asthma but asthma does not have a causal effect on obesity. For this reason, obesity was chosen as the exposure for this study and asthma as the outcome.

3.4 Why this study is important

The relationship between obesity and asthma is an important one as many individuals are affected by one or both of these conditions. While there have been many studies conducted on the relationship between obesity and asthma, this study aims to show how published data can be used to identify relationships and causality between two conditions. The use of both a systematic review and Mendelian randomisation to identify relationships between an exposure and outcome of interest is a useful tool. It provides information not only on the association but also on the direction of causality in the relationship. This study aims to demonstrate methods which make use of available summary statistics. This use of summary statistics makes this form of research more accessible and applicable (and the results often more reliable) than most forms of research using original data. The statistical methods implemented in this review will be done using a frequentist and Bayesian approach. This will provide a demonstration of how these methods can be used by either statistical paradigm. This will make the methods accessible to individuals who prefer one paradigm over the other and will also provide a critical comparison of the results obtained using the two paradigms. Bayesian methods are becoming increasingly popular in the statistics community which makes it meaningful to implement statistical methods using both the more customary frequentist approach and the Bayesian approach.

4 Systematic Review

4.1 Introduction

A systematic review involves collecting all the relevant literature on a chosen topic, sorting through studies based on a set of inclusion and exclusion criteria and then summarizing the results from the remaining studies. For systematic reviews where the topic is medical of nature, the *Cochrane Handbook for Systematic Reviews of Interventions* is a useful guideline for systematic reviews of an intervention (Higgins et al., 2022). This guideline was used in this study to analyse the relationship between obesity and asthma amongst individuals of all ages and ethnic backgrounds.

4.2 Methods for Systematic Review

The methods used in this review follow the outline of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins et al., 2022). A protocol was first created for the review, outlining important criteria for the review. These criteria are summarized in the methods section below.

4.2.1 Criteria for considering studies for this review

Due to the nature of the exposure of the review (obesity), it is not possible to conduct randomized-controlled trials (RCTs). Therefore, the studies that were used were case-control studies and cohort studies as these are the next best thing in terms of the reliability of the results. This implies that the type of data used in the studies will be observational. This means that a causal relationship cannot be identified from this review as this would require RCTs on the topic. However, the review will still attempt to identify a relationship between asthma and obesity.

There were no requirements regarding the type of participants included in the studies. Studies with participants of all ages and ethnic backgrounds, as well as both sexes were included. Studies were not excluded due to underlying health conditions or other characteristics of the participants. This allows an assessment of the relationship between asthma and obesity in a variety of populations.

Studies with duplicate data (more than one study using the same datasets) were excluded as they would produce the same results which would result in the same results being included in the review twice. So the cohorts and datasets used in the studies were screened to avoid this. For the meta-analysis the measures summarising the exposure-outcome association will be restricted to odds ratios as this is the most common association measure. However, initially for the systematic review studies with any appropriate measures will be included.

For the purpose of this review, studies on Obesity and Asthma will be restricted to those published between 2011 and 2022. This is because there have already been reviews produced on this relationship, but this period has not been covered well. Pei-Ching Liu et. al. (2013) did an extensive review on the association between obesity and asthma in children which covered the period from 1966 to 2011.

This review will only use studies published in English as mistranslation of studies published in other languages could introduce error into the review. This review will only include published studies as they are the most accessible. We do note that this could lead to publication bias but the results will be screened for possible publication bias during the meta-analysis section.

4.2.2 Search methods for identification of studies

The search for studies for a systematic review should be as extensive as possible. This ensures the reviews contains as much information as possible and also reduces the risk of reporting bias (Higgins et al., 2022). The choice of databases to search should be research topic specific (ie. medical studies should include medical databases)(Higgins et al., 2022). For reviews of a medical intervention (or exposure) the *Cochrane Handbook for Systematic Reviews of Interventions* recommends using MEDLINE (or Pubmed), Embase and CENTRAL databases if the reviewers have access to these resources (Higgins et al., 2022). However,

Pubmed is the only one that is required as it is a free resource. Ideally unpublished studies should be included in the review(Higgins et al., 2022). However, these studies are difficult to find and are often missing information, which can make this challenging. Another recommendation is to make use of a healthcare librarian or information specialist, however, this is beyond the scope of this review and was not implemented here (Higgins et al., 2022). It is also recommended to create a protocol and search plan before conducting the search to outline all the details of the search (Higgins et al., 2022). This was done for this reviews.

Due to the medical nature of the relationship under investigation, Pubmed was the main database used to search for studies. This is because this is an unfunded review and Pubmed is the only free recommended resource. An internet search (Google Scholar) was also conducted to possibly find additional publicly available papers that were not in the databases. However, this was limited to free research papers (and did not yield results different to those already identified on Pubmed). The reference lists of other reviews were also searched for relevant studies to include.

Terms to be used for the search

- "Obesity and asthma"
- "Asthma and obesity in adults"
- "Asthma and obesity in children"
- "Asthma risk factors"

No resources other than those described above were used for this review.

4.2.3 Data collection and analysis

Studies for inclusion were identified individually by both authors. The selected studies were compared and any discrepancies debated. If a conclusion was not reached, the protocol was to consult supervisors, although this did not occur. Once both parties agreed on a set of initial studies to be included, a more in-depth analysis of the studies was conducted. At this stage any studies that, upon closer examination, no longer met the criteria were excluded and the results of the studies were recorded. The results were then summarised in the systematic review section and then all results that met the criteria of the meta-analysis were used in that section. The data extracted from the studies included outcome data as well as study characteristics and summaries. To assess whether the studies met the inclusion criteria of the meta-analysis, the studies were reviewed individually again by both authors.

Assessment of risk of bias in included studies

Both authors independently assessed the risk of bias for each individual included study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins et al., 2022). Disagreements were resolved through a discussion. The risk of bias was assessed according to the domains of:

- Bias due to confounding. This is bias that arises due to external variables that could influence the intervention/exposure, outcome or both. These variables can bias the relationship between the intervention/exposure and outcome which can cause problems with the reliability of model results.
- Bias in selection of participants into the study. This refers to any bias that can arise due to inclusion or exclusion of participants from the study. It focuses on whether participants were selected after the start of intervention/exposure and the effect this could have on the intervention/exposure or outcome.
- Bias in classification of intervention/exposure. This refers to bias that can arise from incorrect classification of the intervention/exposure group. This can occur when intervention/exposure groups are not clearly defined or when the definition or classification of the intervention/exposure is not consistent.
- Bias due to deviations from intended intervention/exposure. This refers to bias that arises when there are deviations from the intervention/exposure that would not be expected in usual practice. For the purpose of this study the only possible deviations would be weight loss.

- Bias due to missing data. This form of bias arises when missing data in studies is not properly dealt with. This is more of a problem when participants are excluded due to lack of information on the intervention/exposure or outcome.
- Bias in measurement of outcomes. This refers to bias that arises when there is inconsistent measurement of the outcome between the intervention/exposure and non-intervention/non-exposure groups. It also included bias that can arise if knowledge of the intervention/exposure can impact the outcome.
- Bias in selection of the reported result. This refers to bias that arises by researchers selectively reporting favourable results. If multiple models are fitted and not all results are reported this can indicate possible selection bias in the results.

Each potential source of bias was graded as a low, moderate, serious or critical risk of bias. This grading involves answering a series of yes or no questions relating to the study and then assessing the overall risk of bias for a category by checking how serious the deviations from the desired answers are. Risk of bias tables which summarise the bias in each study can be found in the appendix. The risk of bias plots can be found in the section below.

Unit of analysis problems

Unit of analysis problems arise when the unit of analysis in a study is not participants, but rather a group or clinic etc (Higgins et al., 2022). It can also occur when there are more than 2 treatment arms (Higgins et al., 2022). Unit of analysis problems are not too problematic as there are methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* to deal with these problems when they arise (Higgins et al., 2022). However, none of these were present in any of the included studies and adjustments were therefore not required.

Dealing with missing data

Missing data in the form of information from the study or missing participant data can be a problem in systematic reviews (Higgins et al., 2022).

Studies with missing information will still be included in the review, as long as the missing information is not essential to understanding the results or methods of the review. None of the included studies were missing important information in these areas. However, almost all the studies did not report standard errors which are needed for the meta-analysis. This problem was dealt with by manually calculating the standard errors for each study from the confidence intervals and is described in detail in the meta-analysis methods. It is possible that some participants could miss a follow-up due to asthma flare-ups which could mean that the missing data is not missing at random. However, most of the studies were not longitudinal so follow-up wasn't a problem. Most studies that made use of questionnaires had missing data but most of the exposure and outcome data was not missing so this missing data is most likely missing at random and not related to the exposure or outcome. The severity of the missing data was assessed individually for each study as part of the bias assessment. Missing data was not imputed as this would involve getting all of the raw data from study authors and is above the scope of this project.

Assessment of heterogeneity

Heterogeneity in a systematic review (or more specifically, a meta-analysis) refers to variability between the study results of the included studies (Higgins et al., 2022). High heterogeneity indicates that the studies are not all in agreement about the relationship under investigation.

To assess heterogeneity, forest plots were inspected and the I^2 statistic was calculated. This is explained further in the meta-analysis methods section. The interpretation of the I^2 statistic chosen was that recommended in *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins et al., 2022) and is given by:

- 0% to 40%: might not be important
- 30% to 60%: may represent moderate heterogeneity

- 50% to 90%: may represent substantial heterogeneity
- 75% to 100%: considerable heterogeneity

Assessment of reporting biases

Reporting bias occurs when the reporting of results is dependant of the magnitude and direction of the outcome measure (Higgins et al., 2022). It is usually when authors use multiple methods or different sets of data and then selectively report favourable results. Reporting bias can also arise due to publication bias (Higgins et al., 2022). This occurs when the publishing of papers or speed at which papers are published depends on the nature and direction of the results (Higgins et al., 2022).

A funnel plot was used to assess possible publication bias (This is described in detail in the meta-analysis results section). Reporting bias by the authors of the study was assessed individually for each study in the bias assessment. This was done by making sure that the results from all reported methods were reported in the study.

Sensitivity analysis

Sensitivity analyses should be conducted when arbitrary decisions or cut-offs are used in the review (Higgins et al., 2022). This involves redoing the review process with different decisions to see if these change the outcome of the review (Higgins et al., 2022).

There were not many arbitrary decisions made during the review process. The types of participants and data were not restricted past what is applicable to the exposure. Due to the multilevel nature of the meta-analysis data a different model (for example fixed effect) would also not be applicable. Therefore sensitivity analyses in these areas were not conducted. A sensitivity analysis was conducted for the choice of within-study correlation used in the meta-analysis. This is explained in more detail in the meta-analysis methods.

4.3 Results from Systematic Review

4.3.1 Description of studies

An electronic search of the search terms on Pubmed (which is the free version of MEDLINE) yielded 40 210 citations. Upon filtering for articles in the date range (2011 - 2022) there were 15 879 citations and 15 851 were initially excluded. The majority of these were excluded for being reviews rather than original research or for not being relevant to the association of interest. After subsequently screening the full-text articles, ten were excluded due to not meeting the inclusion criteria. These criteria included being within the specified date range (2011-2022), being original research and not reviews, being published in English and being relevant to the association of interest (association between obesity and asthma).Studies that reported the correct outcome measure (Odds Ratio) on the relationship of interest (Obesity and asthma prevalence) were included in the meta-analysis. Six studies met these criteria.

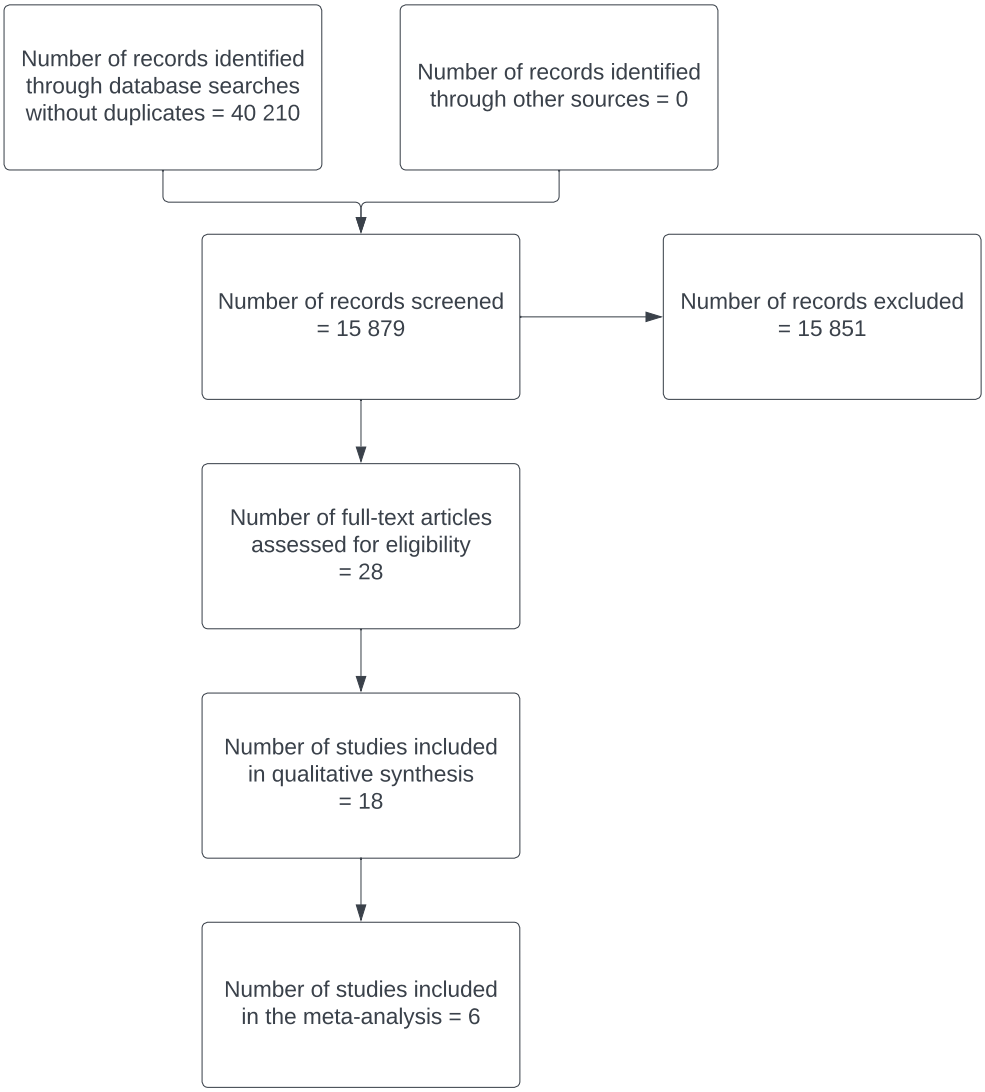


Figure 1: Diagram of the study flow from the search to the meta-analysis.

Included studies

For the systematic review 18 of the 28 studies were included. The included studies and a summary of their characteristics can be found in the Characteristics of included studies section. The included studies all assessed the association between obesity and either asthma prevalence, control or severity, as this is the interest of this review (particularly asthma prevalence). They include cross-sectional and longitudinal study designs and all studies were published between 2011 and 2022. The included studies are also all original research and not review papers.

For the meta-analysis 6 of the 18 studies from the systematic review were included. These studies were: Lu et al. (2016), Ho et al. (2011), Barros et al. (2016), Gonzalez-Barcala et al. (2016), Jeong et al. (2017) and Wang et al. (2015). These studies met the inclusion criteria for the meta-analysis and further information on this can be found in the meta-analysis section below. The meta-analysis is focused on obesity and asthma prevalence, so most studies were excluded for assessing other obesity-asthma associations.

Excluded studies

Ten studies were excluded from the systematic review. Rastogi et al. (2012) was excluded as it measured the association between obesity and various measures that were only loosely associated with asthma severity. These measures were not really the interest of this review. Michelson et al. (2009), Chen et al. (2006) and Tonorezos et al. (2008) were all published before the time range included in this review. These studies slipped through in the original screening and were later identified and excluded. Leija-Martínez et al. (2019) was excluded due to not being relevant to the topic of this review. This was a genetics study on possible epigenetic mechanisms that modify the obesity-asthma relationship. It was also a review and not original research. Baltieri et al. (2022) was excluded due to not being relevant to the topic of this review. This study was investigating the correlation between levels of adipokines and inflamatory mediators with lung function in obese individuals. Zhu et al (2019) was excluded due to not being relevant to the topic of the review. It explores the shared genetic links between obesity-related traits and asthma subtypes in the UK Biobank. This is possibly more relevant for the Mendelian randomisation but not for the systematic review. van Huisstede et al. (2013) was excluded due to not being relevant to the review. It focused on the underdiagnosis and overdiagnosis of asthma in the morbidly obese. Wong et al. (2022) and Oudjedi et al. (2020) were excluded due to being reviews and not original research.

Twelve studies were excluded from the meta-analysis. Many of these studies were excluded due to not reporting the outcome measure of interest (Odds Ratio) or the relevant information to calculate an Odds Ratio. Many studies were also excluded due to focusing on the relationship between obesity and asthma severity or control rather than prevalence. These studies only included participants who already had asthma and therefore did not have a category on no asthma and could not be used for the meta-analysis.

4.3.2 Risk of bias in included studies

The ROBINS-I tool was used to assess risk of bias in included studies (Sterne et al., 2016. However, this tool is intended for non-randomized studies of interventions. The studies included in this review are studies of an exposure and not an applied intervention. The tool was therefore used as a rough guide for the assignment of bias ratings.

For details on risk of bias of included studies, look at the Characteristics of included studies table. For an overview of review authors' judgements about each risk of bias item for each paper and across all papers, see Figure 2 and Figure 3 below. There are no strict rules for the assignment of the overall risk of bias rating to a study. However, to ensure consistency in the ratings, the following guideline was used for rating overall bias:

- Low bias - No critical or serious bias ratings and up to 2 moderate ratings, the rest must be low.
- Moderate - no critical and either 1 serious and one or fewer moderate ratings, or more than 2 moderate ratings and the rest low.
- Serious - either 1 critical and the rest low, or 1 serious and two or more moderate, or 2-3 serious and the rest low.
- Critical - either 1 critical and 1 or more moderate, or 2 or more critical.

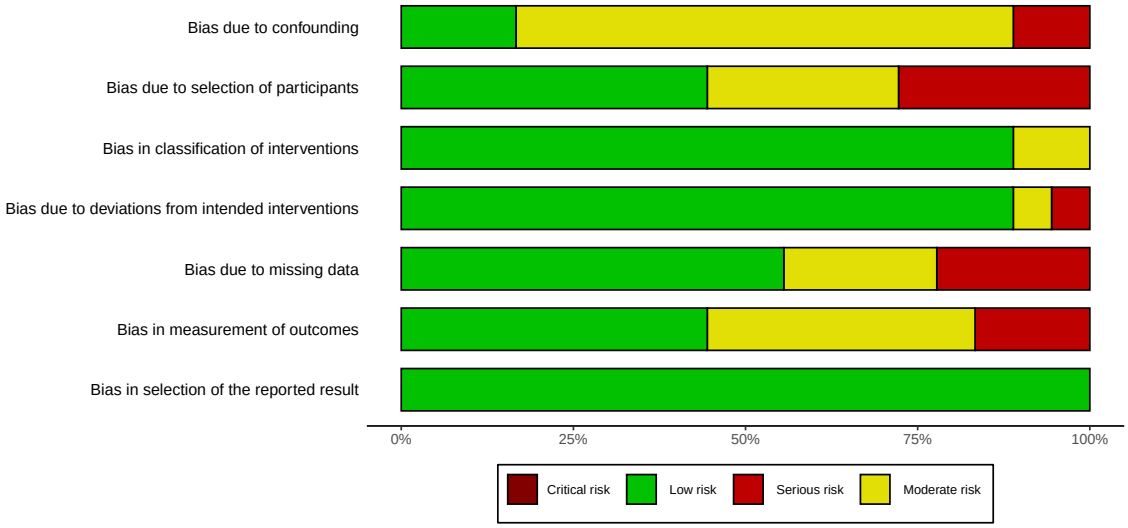


Figure 2: Plot of the overall risk of bias for each category across studies.

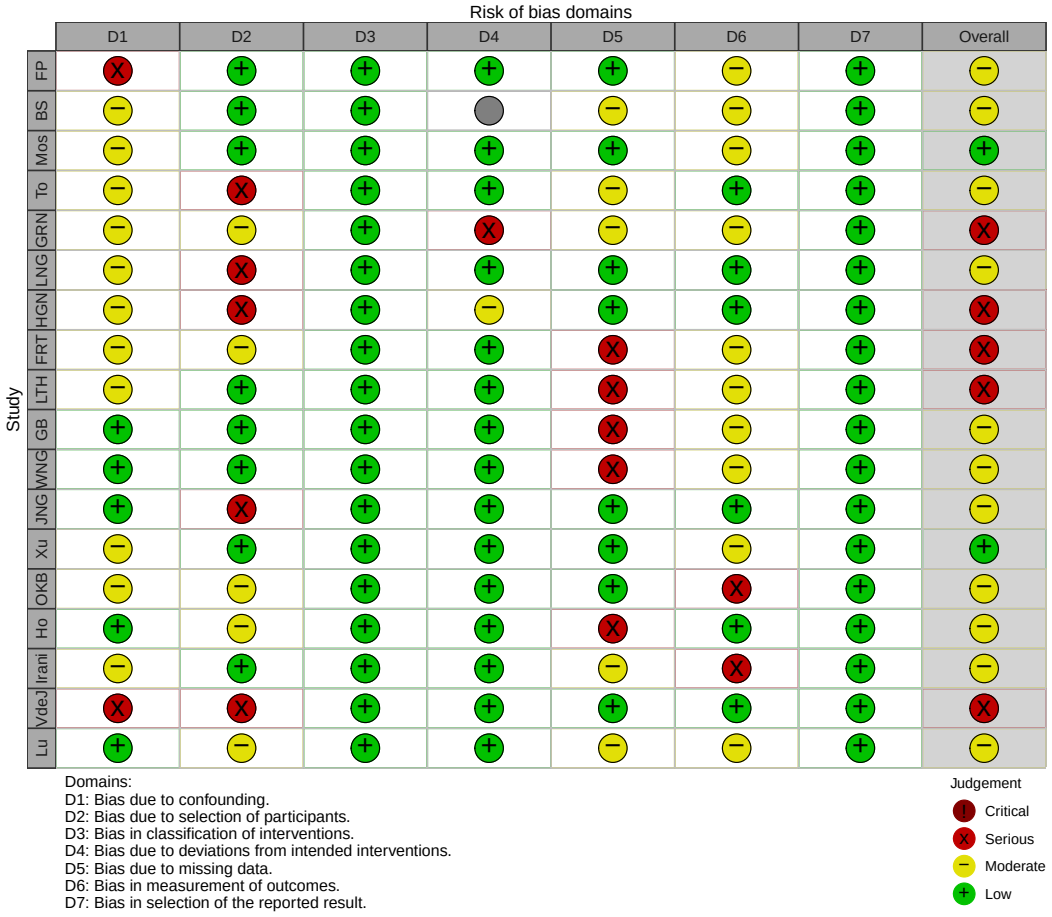


Figure 3: Traffic plot of the risk of bias for each study with respect to each bias domain.

Bias due to confounding

Bias due to confounding can arise when there are variables that affect the relationship between the exposure and outcome. These variables alter the relationship between the exposure and outcome and can make the relationship seem more significant that it probably is. Of the eighteen studies included in the systematic review, two had a serious risk of bias due to confounding (Fitzpatrick et al., 2021; Viana de Jesus et al., 2017), eleven had a moderate risk of bias (Barros et al., 2016; Mosen et al., 2008; To et al., 2018; Green, 2012; Lang et al., 2011; Holguin et al., 2011; Forte et al., 2013; Luthe et al., 2018; Xu et al., 2016; Okubo et al., 2016; Irani et al., 2019), and five had a low risk of bias (Gonzalez-Barcala et al., 2012; Wang et al., 2015; Jeong et al., 2017; Ho et al., 2011; Lu et al., 2017). Most studies accounted for baseline confounding, but few accounted for time-varying confounding when applicable. The most common method applied across studies was an adjusted logistic regression model to account for measured confounders. Since mitigating unmeasured confounding in observational studies is impossible, none of the studies were able to do this. Studies that did not account for confounding in the models used but followed correct procedures so as to not introduce additional possible confounding variables were awarded a serious rating. Studies that accounted for confounding in the models but fell short in one other aspect related to confounding were awarded a moderate rating. Studies that accounted for confounding in the models and did not fall short in any other aspects related to confounding were awarded a low rating.

Bias in the selection of participants into the study

Selection bias is when the exclusion of some eligible participants occurs in a way that leads the association between the exposure and outcome to differ from the observed association in the target trial (an equivalent randomised control trial).

Eight of the studies (Fitzpatrick et al., 2021; Barros et al., 2016; Mosen et al., 2008; Luthe et al., 2018; Gonzalez-Barcala et al., 2012; Wang et al., 2015; Xu et al., 2016; Irani et al., 2019) showed a low risk of bias in this domain because of the inclusion of all participants who would have been eligible for the target trial. Also, for each participant, the start of follow-up and exposure coincided.

The five studies (Green, 2012; Forte et al., 2013; Okubo et al., 2016; Ho et al., 2011; Lu et al., 2017) with a moderate risk of bias received that judgement because selection into the study may have been related to the exposure and outcome. However, the authors either used appropriate methods to adjust for the selection bias, or the start of the exposure did not coincide for all participants, and the proportion of participants for which this was true was too low to induce important bias.

The five studies (To et al., 2018; Lang et al., 2011; Holguin et al., 2011; Jeong et al., 2017; Viana de Jesus et al., 2017) with a serious risk of bias received that judgement because selection into the study was related (but not very strongly) to exposure and outcome but could not be or was not adjusted for. Another reason was that the start of the exposure did not coincide for all participants, and a potentially important amount of follow-up time was missing from the analyses.

Bias in the classification of interventions/exposures

Misclassification of the exposure status may introduce bias. Misclassification is seldom a problem in randomised control trials because a crucial feature of the study is that researchers actively assign interventions (Sterne et al., 2016). However, information about interventions/exposures allocated or received in observational studies needs to be collected, which could be a source of bias. All included studies (Fitzpatrick et al., 2021; Barros et al., 2016; Mosen et al., 2008; To et al., 2018; Green, 2012; Lang et al., 2011; Holguin et al., 2011; Forte et al., 2013; Luthe et al., 2018; Gonzalez-Barcala et al., 2012; Wang et al., 2015; Jeong et al., 2017; Xu et al., 2016; Okubo et al., 2016; Ho et al., 2011; Irani et al., 2019; Viana de Jesus et al., 2017; Lu et al., 2017) had a low bias in this domain because the exposure status was well defined.

Bias due to deviations from intended interventions/exposures:

This domain considers biases that arise when there are systematic deviations from the intervention/exposure that a participant is assigned to. It also accounts for differences in care between the intervention and non-intervention groups. (Sterne et al., 2016). The exposure of interest is obesity so deviations would include

obese participants losing weight (and changing BMI classification) over the course of the study or non-obese participants gaining weight (and changing BMI classification) over the course of the study. With regards to the included studies, fifteen of the eighteen studies (Fitzpatrick et al., 2021; Mosen et al., 2008; To et al., 2018; Lang et al., 2011; Forte et al., 2013; Luthe et al., 2018; Gonzalez-Barcala et al., 2012; Wang et al., 2015; Jeong et al., 2017; Xu et al., 2016; Okubo et al., 2016; Ho et al., 2011; Irani et al., 2019; Viana de Jesus et al., 2017; Lu et al., 2017) showed low bias. This is because most of the included studies are cross-sectional and not longitudinal, therefore, there is not a possibility to deviate from the exposure. One study (Green, 2012) was given a serious bias judgement because deviations from usual practice were unbalanced between the exposure groups, which was likely to have affected the outcome. This involved participants changing weight over the course of the study. The study with moderate bias (Holguin et al., 2011) had deviations from usual practice, but their impact on the outcome was expected to be slight. This involved participants changing weight over the course of the study, however, it was only very few participants and was roughly balanced between exposure groups.

Bias due to missing data

Missing data may arise, among other reasons, through attrition (loss to follow up), missed appointments, incomplete data collection and participants being excluded from analysis by primary investigators (Sterne et al., 2016). In NRSI, data may be missing for baseline characteristics (including interventions received or baseline confounders), outcome measurements, other variables involved in the analysis or a combination of these (Sterne et al., 2016). The majority of the included studies had a low risk of bias (Fitzpatrick et al., 2021; Mosen et al., 2008; Lang et al., 2011; Holguin et al., 2011; Jeong et al., 2017; Xu et al., 2016; Okubo et al., 2016; Viana de Jesus et al., 2017) because data were complete, proportions of and reasons for missing participants were similar across exposure groups, or the analysis addressed missing data and likely removed any risk of bias. Five studies had a serious risk of bias (Forte et al., 2013; Luthe et al., 2018; Gonzalez-Barcala et al., 2012; Wang et al., 2015; Ho et al., 2011) because the proportions of missing participants differed substantially across exposures, or reasons for missing data differed substantially across exposures. The five studies (Barros et al., 2016; To et al., 2018; Green, 2012; Irani et al., 2019; Lu et al., 2017) that showed a moderate amounts of bias had proportions and reasons for missing participants that differed slightly across exposure groups, and it is unlikely that the analysis would have removed the risk of this bias arising from the missing data.

Bias in the measurement of outcomes

Bias may be introduced if outcomes are misclassified or measured with error (Sterne et al., 2016). With regards to the studies, six had a low risk of bias (To et al., 2018; Lang et al., 2011; Holguin et al., 2011; Jeong et al., 2017; Ho et al., 2011; Viana de Jesus et al., 2017), ten had a moderate risk of bias (Fitzpatrick et al., 2021; Barros et al., 2016; Mosen et al., 2008; Green, 2012; Forte et al., 2013; Luthe et al., 2018; Gonzalez-Barcala et al., 2012; Wang et al., 2015; Xu et al., 2016; Lu et al., 2017), and two had a serious risk of bias (Okubo et al., 2016; Irani et al., 2019).

The studies that had a low risk of bias received that judgement because the methods of outcome assessment were comparable across exposure groups. The outcome measure was unlikely to have been influenced by knowledge of the exposure received by study participants, or the outcome assessors were unaware of the exposure received by study participants. Any error in measuring the outcome was also unrelated to the exposure status.

Studies with a moderate risk of bias (Fitzpatrick et al., 2021; Barros et al., 2016; Mosen et al., 2008; Green, 2012; Forte et al., 2013; Luthe et al., 2018; Gonzalez-Barcala et al., 2012; Wang et al., 2015; Xu et al., 2016) differed from those with low bias because there were more instances or possibilities for error in the measurement of outcomes. However, this error in measuring the outcome was only minimally related to exposure status. Many of the studies that received this bias rating made use of survey type questions to record asthma status, which introduces an element of possible error to the measurement. The studies that received a serious judgement received it because the outcome measure was subjective, and the outcome was assessed by assessors aware of the exposure of study participants.

Bias in the selection of the reported result

Selective reporting within clinical trials can be described as the failure to report, or partial reporting of, outcome domains that were measured and analysed (Sterne et al., 2016). An outcome domain is defined as a valid state or endpoint of interest, irrespective of how it is measured, an outcome measurement as a specific measurement made on the study participants, and an outcome analysis as a specific result obtained by analysing one or more outcome measurements (Sterne et al., 2016). Every study (Fitzpatrick et al., 2021; Barros et al., 2016; Mosen et al., 2008; To et al., 2018; Green, 2012; Lang et al., 2011; Holguin et al., 2011; Forte et al., 2013; Luthe et al., 2018; Gonzalez-Barcala et al., 2012; Wang et al., 2015; Jeong et al., 2017; Xu et al., 2016; Okubo et al., 2016; Ho et al., 2011; Irani et al., 2019; Viana de Jesus et al., 2017; Lu et al., 2017) showed a low risk of bias for this domain because there was clear evidence (from the statistical analyses) that all reported results correspond to all intended outcomes, analyses and sub-cohorts, where applicable.

4.3.3 General overview of the relationship between obesity and asthma

All relevant statistics from the studies discussed below can be found in Table 1. Lang et. al. (2011) was not included in the table as this study reports too many asthma measures. The paper can be found in the references.

Among the 18 studies included in the qualitative analysis, 17 found that obesity was positively associated with either asthma prevalence or poorer asthma outcomes in at least some groups of individuals. One study (Xu et al., 2016) found that there was no relationship between obesity and asthma prevalence or outcomes. One study (Green et al., 2012) used asthma as their exposure and obesity as their outcome. However, the study does not use valid methods to determine causality so this is still just and association between obesity and asthma.

The positive association differed between the sexes, with females showing a greater association than males for many of the studies. Most of the studies used BMI (age-specific and sex-specific where necessary according to WHO) to classify overweight and obese participants. Most participants with asthma across studies were clinically diagnosed with the disease and questionnaires such as the standardised Asthma Control Questionnaire (ACQ), Asthma Therapy Assessment Questionnaire and Paediatric Asthma Quality of Life Questionnaire (PAQLQ) were mostly used to determine severity of asthma symptoms.

Eleven of the 18 included studies adopted a cross-sectional design. Nine confirmed a positive relationship between obesity and either asthma prevalence or poorer asthma outcomes, especially among women and children, indicating that sex and age play a significant role in the strength of the association. Six of the studies used a longitudinal design and of these, five identified a positive relationship between obesity and either asthma prevalence or poorer asthma outcomes. Many of these longitudinal studies also found that the association between obesity and asthma became weaker as age increased.

Many of the studies accounted for the following possible confounders in some form or another: age, gender and physical activity level. Some other common confounders included by some studies were: race/ethnicity, family history of asthma, family income, education, pet ownership, presence of household mould, smoking, second-hand smoke exposure, educational status, dietary patterns, oral corticosteroid use in the past month, evidence of gastroesophageal reflux disease (GERD) and inhaled corticosteroid use.

A few studies identified age as an effect modifier instead of as a confounder to make the interaction between obesity and asthma phenotype clearer and concluded that age is a significant effect modifier, with an increase in age reducing the influence of obesity on asthma, in general. Sex was also used as an effect modifier by a few of the included studies and these found a significant effect of sex, with females being at higher risk of the obesity-asthma interaction.

4.3.4 Relationship difference by age

Three studies identified in the search and included in the review address the differences in the relationship between obesity and asthma by age group.

Gonzalez-Barcala et. al. (2013) was a cross-sectional study which included 7485 children aged 6-7 years and 8496 adolescents aged 13-14 years. The data was gathered through a questionnaire which recorded

answers to questions about wheezing ever, current asthma, exercise-induced asthma and asthma severity. The questionnaire also recorded information about possible confounding variables such as owning pets and parental asthma. The parents of the children in the 6-7 age group filled in the questionnaire and the 13-14 year old group filled in the questionnaire themselves. Overweight and obesity were determined using BMI. Multiple logistic regression was used to obtain prevalence odds ratios for each of the groups for the four outcome variables (wheezing ever, current asthma, exercise-induced asthma and asthma severity). These odds ratios were adjusted for possible confounders including: gender, dog or cat in the home, parental smoking, parental asthma, and maternal education. The study found that obesity was associated with and increase in wheezing and exercise-induced asthma in the children aged 6-7 but there was no significant association in the 13-14 age group. This indicates that there is a possible impact of age in the relationship between asthma prevalence and obesity and that in younger children obesity results in a higher prevalence of asthma but not in adolescents. However, it is important to note possible limitations of this study which include that the asthma was not diagnosed by a medical professional and was rather assessed by the responses to questions about wheezing in the questionnaire. Another limitation is that the parents answered the questionnaire in the younger age group and the children themselves in the older age group. This could introduce some error and inconsistencies in the responses between groups.

Holguin et. al (2011) was a retrospective longitudinal cohort study which included individuals from the SARP study that met the asthma criteria (this involved either a 2% increase in FEV after a short-acting bronchodilator or a 20% decrease in FEV after in-halation of methacholine). There were 1049 participants who were all 18 years or older. The participants were split into two groups, those with asthma onset at 12 years and older and those with onset under 12 years. Various asthma symptoms and measures of severity were the outcome variables and these were compared between the normal weight, overweight and obese groups for each of the age groups using univariate and multivariate logistic regression. For each outcome variable an odds ratio was reported for each weight category and for each of the two age groups. These odds ratios were adjusted for confounders including age, sex, race, atopy, and asthma duration. Obese participants with early-onset asthma had more airway obstruction, bronchial hyperresponsiveness and higher odds of ever having 3 or more previous oral steroid tapers per year or intensive care unit admissions for asthma per preceding year. In the participants with early-onset asthma there was also a relationship between increasing BMI and the duration of asthma. Obesity seemed to be related to various measures of asthma severity in both the early and late-onset groups, however, this relationship seemed stronger in the early-onset group.

Lang et. al. (2011) was a longitudinal cohort study which included 490 patients with mild persistent asthma. Obesity was categorised by BMI and the outcome variables were asthma characteristics such as spirometry, asthma control, airway pH, and perception of airflow changes. Initially a longitudinal multivariate mixed-effect model was fitted without stratification for age. This showed no significant association between obesity and asthma characteristics. The model was then stratified by age and confounders such as gender, race and atopy were accounted for. The four age groups used were: 6-11 years, 12-17 years, 18-44 years and 45-76 years. In the 6-11 years age category the effect of obesity on reduction in lung function was the largest but in this age group non-obese children reported more asthma symptoms that obese children. Obese 12-17 year olds has greater airflow obstruction and more asthma symptoms that non-obese 12-17 year olds. Both of the adult age categories showed no significant differences in asthma characteristics between obese and non-obese individuals overall. However, in the 12-17 and 18-44 year groups there was greater obesity-related asthma impairment in females. This study showed that the impact of obesity on the asthma phenotype is reduced with increasing age.

All three of these studies indicate that the relationship between obesity and asthma prevalence and severity is modified by age. They all agree that as age increases or age of asthma onset increases, that the impact of obesity on asthma is decreased.

4.3.5 Relationship difference by sex

Wang et. al. (2015) is a cross sectional study that included 33,153 adults (4197 of which had asthma). The data came from the 2012 National Health Interview Survey. Obesity was determined by BMI and was classified into classes: class 1 was defined as BMI 30-34.9, class 2 as BMI 35-39.9, and class 3 as BMI 40

or over. The outcome was asthma prevalence which was determined by the survey question: "Has a doctor or health care professional ever told you had asthma?". Multiple logistic regression was used to examine the joint relationship between obesity and sex on asthma prevalence. The model was adjusted for age, race, marital status, smoking status, hay fever, stress, skin allergy, depression, and insomnia. Class 1, 2 and 3 obesity was associated with asthma prevalence. There was a stronger association between class 2 obesity and asthma in women than in men. However, the sex difference in the association between BMI and asthma overall was not significant. This indicates that sex is something to be considered when modelling the obesity-asthma relationship but does not present strong evidence of a difference in the relationship between obesity and asthma prevalence.

Lu et. al. (2016) is a cross-sectional study that included 4828 participants. The data came from The National Health and Nutrition Examination Survey (NHANES). The participants in this survey are disproportionately adolescents and African-American and Mexican-American populations. Obesity was measured using BMI percentiles adjusted for age. Asthma was assessed by asking participants if they had ever been diagnosed with asthma by a healthcare professional and if participants answered yes, there were additional questions about severity and current asthma. Logistic regression was used to examine the relationship between obesity and asthma stratified by sex. The model was adjusted for age, survey year, poverty index ratio, second hand smoke exposure, race/ethnicity, and fitness groups. There was a positive relationship between obesity and history of asthma and obesity and current asthma in girls but neither of these relationships were present in boys. This indicates relatively strong evidence for a difference in the relationship between obesity and asthma prevalence for boys and girls.

Lang et. al. (2011), outlined in the section above on age, reported on the difference in the relationship between obesity and asthma severity between males and females. They found in the 12-17 and 18-44 year groups there was greater obesity-related asthma impairment in females than in males.

4.3.6 Relationship differences between countries

While there is a lack of research directly comparing the differences or similarities between the obesity-asthma relationship in different countries or areas, there are a few different countries represented in the included studies. The majority of the study populations come from the United States, however, there are also populations from Lebanon, Brazil, Taiwan, Portugal, Japan, Spain, and Switzerland.

Ten of the included studies make use of US populations. Nine of these studies conclude that there is a relationship between obesity and an increase in either asthma severity or prevalence, or poorer asthma control. Some studies found stronger and more consistent relationships than others but 9 of the studies reported a significant relationship. This is strong evidence for a relationship between obesity and asthma in US populations. The only study that did not report a significant relationship was Xu et. al. (2016) which found no significant relationship between obesity and asthma control once they adjusted their model for confounders.

Two of the included studies use Brazilian populations. Viana de Jesus et. al. (2017) found a relationship between obesity and poorer asthma control. It is important to note, however, that this study made use of mostly summary statistics and Chi-squared and Kruskal-Wallis test of differences between groups. They fitted no models to the data and therefore did not account for any confounders of this relationship. Therefore, the validity of this relationship is very questionable. Carra Forte et. al. (2013) found no relationship between obesity and asthma control, as well as no relationship between obesity and asthma severity. The study did find a relationship between obesity and higher FEV percentage. The results of these two papers are somewhat contradictory and more research is required in Brazil to clarify this relationship.

Irani et. al. (2019) made use of a Lebanese population. This study aimed to determine if there was a relationship between obesity and asthma control. It involved participants with asthma filling in the Asthma Control Test questionnaire and BMI was measured. Using logistic regression, they found that obese patients has significantly poorer asthma control than non-obese patients. This provides some evidence that obesity leads to poorer asthma control in Lebanese populations. More research is required to confirm this

relationship and also to provide insight into the relationship between obesity and asthma prevalence and severity in Lebanon.

Gonzalez-Barcala et. al. (2013), which has already been discusses in depth, made use of a Spanish population for their study. As mentioned above there was a relationship that was identified between obesity and asthma prevalence, however, only in children and not adolescents.

Barros et. al. (2016) used a Portuguese population for their study. The study aimed to look at the relationship between obesity and asthma prevalence, incidence and severity. Obesity was classified into 4 classes and asthma was diagnosed by a medical professional. Using logistic regression, they found a relationship between all classes of obesity and asthma ever, current asthma, current persistent asthma and current severe asthma.

To et. al. (2018) used a Japanese population to investigate the relationship between obesity and asthma control. They noted that other studies had shown that obesity characteristics differ between males and females and therefore split their population into males and females to look at the relationship separately. Using multivariate logistic regression they found no association between obesity and any measures of asthma control in males but they did find a relationship between obesity and frequent asthma exacerbations.

Jeong et. al. (2017) made use of latent class analysis to identify different asthma classes in their Swiss population. They then used these classes to see if there was a relationship between obesity and any of the asthma classes in the population. The four asthma classes identified were persistent multiple symptom-presenting asthma, symptom-presenting asthma, symptom-free atopic asthma, and symptom-free non-atopic asthma. They found an association between obesity and the two classes of symptomatic asthma but found no relationship between obesity and asymptomatic asthma.

Ho et. al. (2011) aimed to asses whether there is a relationship between obesity and people with pre-asthmatic symptoms developing asthma in a Taiwanese population. There was an initial baseline meeting and then a 12 month follow up to assess whether the pre-asthmatic symptoms developed into asthma or not. Using logistic regression, they found that in girls there was a significant relationship between being overweight and developing asthma but not for obesity. In boys there was no observed relationships.

None of the studies identified for this review include populations from any African countries. Therefore no comments can be made about the relationship in African populations.

Table 1: Summary of Statistics in all included studies. Standard errors are only reported for studies that meet the criteria for the meta-analysis.

Study	**Outcome measure**	**Relationship**	**Result (OR in most studies)**	**Standard error of log(OR)**	**p-value**	**Confidence interval**
Lu et. al.	Odds Ratio	Obesity and history of asthma in girls	1.63	0.1746	not reported	1.16, 2.3
	Odds Ratio	Obesity and history of asthma in boys	0.96	0.1749	not reported	0.68, 1.35
	Odds Ratio	Obesity and current asthma in girls	1.73	0.2165	not reported	1.13, 2.64
	Odds Ratio	Obesity and current asthma in boys	0.90	0.2168	not reported	0.59, 1.38

Ho et. al.	Odds Ratio	Obesity and developing asthma in 12 month period in girls	1.75	0.2025	0.01	1.18, 2.61
	Odds Ratio	Obesity and developing asthma in 12 month period in boys	1.04	0.2081	0.86	0.69, 1.56
Irani et. al.	Odds Ratio	BMI and good asthma control in males	0.84		0.001	0.76, 0.93
	Odds Ratio	BMI and good asthma control in females	0.53		0.078	0.26, 1.07
Gonzalez-Barcala et. al.	Odds Ratio	Obesity and wheezing ever in 6-7 year olds	1.35	0.1050	not reported	1.10, 1.66
	Odds Ratio	Obesity and current asthma in 6-7 year olds	1.31	0.1465	not reported	0.98, 1.74
	Odds Ratio	Obesity and severe asthma in 6-7 year olds	1.20		not reported	0.74, 1.94
	Odds Ratio	Obesity and exercise-induced asthma in 6-7 year olds	1.62		not reported	1.10, 2.36
	Odds Ratio	Obesity and wheezing ever in 13-14 year olds	0.62	0.3064	not reported	0.34, 1.13
	Odds Ratio	Obesity and current asthma in 13-14 year olds	0.52	0.1050	not reported	0.22, 1.21
	Odds Ratio	Obesity and severe asthma in 13-14 year olds	0.86		not reported	0.31, 2.41
	Odds Ratio	Obesity and exercise-induced asthma in 13-14 year olds	1.04		not reported	0.60, 1.79
Barros et. al.	Odds Ratio	Class 1 and 2 obesity and asthma ever	1.39	0.0092	not reported	1.36, 1.41
	Odds Ratio	Class 3 obesity and asthma ever	3.24	0.0252	not reported	3.08, 3.40
	Odds Ratio	Class 1 and 2 obesity and current asthma	1.86	0.0110	not reported	1.82, 1.90
	Odds Ratio	Class 3 obesity and current asthma	4.73	0.0264	not reported	4.49, 4.98

Mosen et. al.	Odds Ratio	Obesity and poor asthma-specific quality of life	2.80		not reported	1.60, 4.90
	Odds Ratio	Obesity and poor asthma control	2.70		not reported	1.70, 4.30
	Odds Ratio	Obesity and history of asthma hospitalisations	4.60		not reported	1.40, 14.40
To et. al.	Odds Ratio	Obesity and frequent asthma exacerbation in females	2.29		0.008	1.24, 4.22
	Odds Ratio	Obesity and frequent asthma exacerbation in males	0.784		0.60	0.32, 1.95
Wang et. al.	Odds Ratio	Class 1 obesity and asthma prevalence overall	1.27	0.0664	not reported	1.11, 1.44
	Odds Ratio	Class 2 obesity and asthma prevalence overall	1.55	0.0867	not reported	1.31, 1.84
	Odds Ratio	Class 3 obesity and asthma prevalence overall	1.85	0.0921	not reported	1.54, 2.21
	Odds Ratio	Class 1 obesity and asthma prevalence in females	1.37		not reported	1.15, 1.62
	Odds Ratio	Class 2 obesity and asthma prevalence in females	1.71		not reported	1.39, 2.10
	Odds Ratio	Class 3 obesity and asthma prevalence in females	2.11		not reported	1.70, 2.63
	Odds Ratio	Class 1 obesity and asthma prevalence in males	1.14		not reported	0.92, 1.41
	Odds Ratio	Class 2 obesity and asthma prevalence in males	1.36		not reported	1.01, 1.82
	Odds Ratio	Class 3 obesity and asthma prevalence in males	1.40		not reported	1.01, 1.96
Luthe et. al.	Odds Ratio	Obesity and mechanical ventilation	1.77		<0.001	1.63, 1.92
	Odds Ratio	Obesity and hospital length of stay $\geq$ 3 days	1.37		<0.001	1.32, 1.43
	Odds Ratio	Obesity and in hospital mortalty	0.99		0.97	0.63, 1.55

Okubo et. al.	Odds Ratio	Obesity and use of mechanical ventilation	1.59		not reported	1.28, 1.99
Xu et. al.	Odds Ratio	Obesity and poor asthma control	1.04		not reported	0.95, 1.14
	Odds Ratio	Obesity and poor Mini-AQLQ	1.09		not reported	0.99, 1.2
Green et. al.	Average partial effect or marginal effect	Obesity and asthma ever	0.09		< 0.01	not reported
	Average partial effect or marginal effect	Obesity and new asthma	0.06		< 0.1	not reported
Jeong et. al.	Odds Ratio	BMI and multiple symptom-presenting asthma	1.32		not reported	1.09, 1.60
	Odds Ratio	BMI and symptom-presenting asthma	1.23		not reported	1.08, 1.41
	Odds Ratio	BMI and symptom-free atopic asthma	1.01		not reported	0.85, 1.19
	Odds Ratio	BMI and symptom-free non-atopic asthma	1.04		not reported	0.88, 1.21
Forte et. al.	Odds Ratio	Obesity and FEV	1.01		not reported	0.99, 1.03
	Chi-squared test of difference between groups	Obesity and asthma control	not reported		0.74	
Holguin et. al.	Odds Ratio	Obesity and asthma severity in late-onset group	1.85		not reported	1.10, 3.00
	Odds Ratio	Obesity and asthma severity in early-onset group	2.10		not reported	1.30, 3.50
Jesus et. al.	Chi-squared test of difference between groups	Obesity and severe asthma	not reported		0.03	
	Kruskal-Wallis test of difference between groups	Obesity and AQLQ score	not reported		< 0.01	
Fitzpatrick et. al.	Chi-squared test of difference between groups	Obesity and AQLQ	not reported		< 0.05	
	Chi-squared test of difference between groups	Obesity and asthma contol	not reported		< 0.05	

5 Meta-Analysis

A meta-analysis involves using summarised data from published studies to investigate a relationship between an exposure/intervention and outcome of interest. It involves fitting a model that can aggregate the evidence from individual studies to produce an overall estimate that summarises the size and direction of the relationship between the exposure and outcome.

A meta-analysis is usually conducted using a fixed or mixed-effect model. A mixed-effect model is the more common choice and is recommended if the objective is to generalise the results of the meta-analysis. A fixed effect model assumes one true underlying effect, which was not the case in the data used for this analysis, because there were subgroups in the data. For this reason, a mixed effect-model was chosen.

All mixed-effect meta-analyses take on a multilevel model structure due to the combining of estimates from multiple studies. The most basic meta-analysis model has a two-level structure, with the first layer accounting for variation between the participants and the second layer accounting for variation between the studies (Harrer et al., 2021).

This mixed effect model can be implemented using a frequentist or Bayesian approach and both have been done here. The Frequentist approach is discussed in full, followed by the Bayesian approach. All analyses in these sections were done using R and all plots were generated in R (R Core Team, 2022).

5.1 Data preparation

The data preparation process is the same for the frequentist and Bayesian approaches. The 18 studies from the systematic review were screened for eligibility for the meta-analysis. The criteria necessary for inclusion were that the relationship being investigated was between obesity and asthma prevalence and that the study reported Odds Ratios (OR) as the effect estimate. This yielded 6 studies for inclusion and the summarised data was extracted from these 6 studies. This involved extracting effect estimates in the form of Odds Ratios (OR), as well as 95% confidence intervals for the estimates. These estimates and confidence intervals were then converted to log(OR)(β coefficients) as this makes the estimates symmetrical and makes the computation simpler. None of the studies reported standard errors so the log standard errors were calculated manually from the confidence intervals using:

$$\ln(se) = \frac{\ln(95\%\text{ CI upper bound}) - \ln(95\%\text{ CI lower bound})}{1.96 \times 2} \tag{1}$$

There were multiple effect sizes extracted for each study as the studies make use of different subgroups in their analyses and, therefore, report more than one estimate.

5.2 Frequentist Approach

5.2.1 Methods

A modification of the two-level mixed effect model involves adding a third level. In this model, the first layer accounts for variation between participants, the second layer accounts for variation between outcomes and the third layer accounts for variation between studies (Harrer et al., 2021). This three-level mixed-effect model was fitted to the data in this analysis to account for the multiple estimates that are reported in each study. Some studies report different estimates for different subgroups of the population which is why there are 15 outcomes but only 6 studies. This three-level model formulation is given by:

$$\theta_{ijk} = \mu + \zeta_{jk} + \zeta_k + \epsilon_{ijk} \tag{2}$$

This can also be represented level by level by:

Level 1 model:

$$\theta_{ijk} = \theta_{jk} + \epsilon_{ijk} \tag{3}$$

Level 2 model:

$$\theta_{jk} = \kappa_k + \zeta_{jk} \tag{4}$$

Level 3 model:

$$\kappa_k = \mu + \zeta_k \tag{5}$$

Where i represents the participants, j represents the outcome and k represents the study. ϵ_{ijk} is the residual (the individual-specific deviation from the outcome's predicted estimate). θ_{jk} is the effect size over outcomes, j and studies, k. This follows a distribution with mean κ_k. ζ_{jk} is the within-study heterogeneity (outcome random intercept). ζ_k is the between-study heterogeneity (study's specific deviation from the overall mean). κ_k is the effect size over the studies and follows a distribution with mean μ. Here μ is the fixed intercept (overall mean).

5.2.2 Statistical Analysis

Model Fitting

This three-level random effects model was fitted to the data for the frequentist approach using the *rma.mv* function from the *metafor* package in R. The significance test was changed from the default z-test to a t-test as this prevents unjustified significant results (Harrer et al., 2021). Restricted Maximum Likelihood Estimation (REML) was used to estimate the model parameters. This is preferred for mixed effect models as it produces unbiased estimates of the variance components. (Meza, Jaffrézic and Foulley, 2007) Due to the correlation between estimates from the same study it is important to use robust variance estimation to generate an estimated covariance matrix for the effect sizes (Harrer et al., 2021). This was done using the *clubSandwich* package. The correlation coefficient (ρ) used was 0.5. This correlation was chosen as a moderate level of correlation as many of the study estimates are of different subgroups of the population. This means that the effect sizes should not be too correlated. A sensitivity analysis was conducted using various levels of ρ and this found that the estimates and p-values associated with the estimates do not change drastically based on the level of ρ used. The results of the sensitivity analysis can be found in Table 2. This indicates that the chosen level of ρ is acceptable as it would not change the model results much if it was slightly inaccurate.

Table 2: Sensitivity analysis model results for different values of ρ

ρ	β coefficient	p-value
0	0.3709	0.0673
0.2	0.3747	0.0667
0.4	0.3783	0.0662
0.5	0.3801	0.0660
0.6	0.3818	0.0658
0.8	0.3850	0.0654
1	0.3880	0.0651

Model Validation

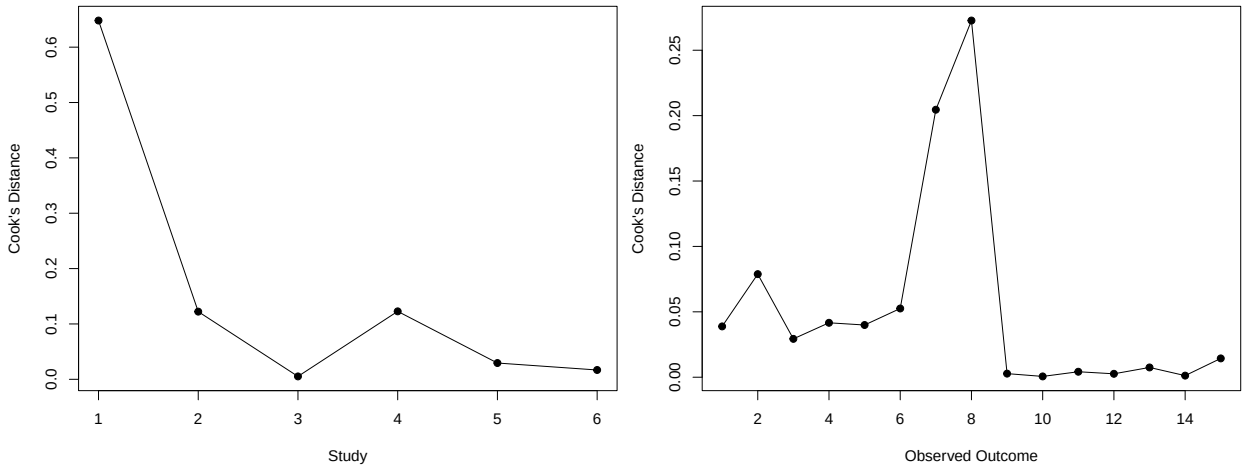
The final 3-level model with robust variance estimation was validated to ensure that underlying model assumptions were met. This involved assessing the residuals, influence and leverage of the observed outcomes.

Figure 4 shows the Cook's distance plotted for each observed outcome (There are 15 outcomes as each study reported more than one effect size) and for each study in the model. Figure 4a indicates that Lu et al. (2016) possibly has high influence compared to the other studies, however, this study is not an outlier and has similar estimates to many of the other included studies. This high influence is therefore, probably not a

problem. Outcomes 7 and 8 show high influence relative to the other outcomes (Figure 4b). These outcome measures are from the Barros et al. (2016) study. Outcome 7 is the association between Class 1 and 2 obesity and asthma. Outcome 8 is the association between Class 3 obesity and asthma. This is a possible problem as these observed outcomes (especially outcome 8) are outliers which can be seen from the forest plot in Figure 7. These outcomes also have high leverage which can be seen from the relatively high hat values in Figure 5. These outcome measures had very low variance and were, therefore, weighted highly in the model due to the weighting method being inverse-variance weighting (This can be seen from the relatively large squares in the forest plot). This would explain the high influence of these outcomes. None of the other observed outcomes seem problematic from the plots in Figures 4-6. A sensitivity analysis was conducted by removing Lu et al. (2015), outcome 7 and outcome 8 from the data individually and rerunning the model without these observations. The results of this sensitivity analysis can be found in Table 3. Removing Lu et al. increases the Odds ratio slightly but this is not too much of a change. This also results in a wider confidence interval and larger p-value. Removing outcome 7 results in a higher Odds ratio with a difference of 0.11 from the original Odds ratio. This also results in a model with wider confidence intervals and a larger p-value. Removing outcome 8 leads to a decrease in Odds ratio by nearly 0.12. This produces a narrower confidence interval and a smaller p-value. This is expected as outcome 8 was quite a large outlier in the data. This confirms that Lu et al. (2015) does not appear to be a problem as it does not change the model estimate by much and is not an outlier. However, it does indicate a possible problem with outcomes 7 and 8 as these do seem to be relatively influential points.

Table 3: Model results of model refitted removing each of the possible high influence studies or outcomes. Outcomes 7 and 8 are the associations for Class 1 and 2 obesity and Class 3 obesity respectively, from Barros et. al. (2016)

Model	**Odds ratio**	**p-value**	**95% Confidence Interval**
Model with no removals	1.462	0.066	0.964, 2.219
Model without Lu et al. (2015)	1.503	0.098	0.888, 2.545
Model without outcome 7	1.572	0.094	0.895, 2.773
Model without outcome 8	1.345	0.062	1.059, 1.709



(a) Cook's Distance plot of the 15 odds ratios

(b) Cook's Distance plot of the 6 studies

Figure 4: Cook's Distance plots assessing influence.

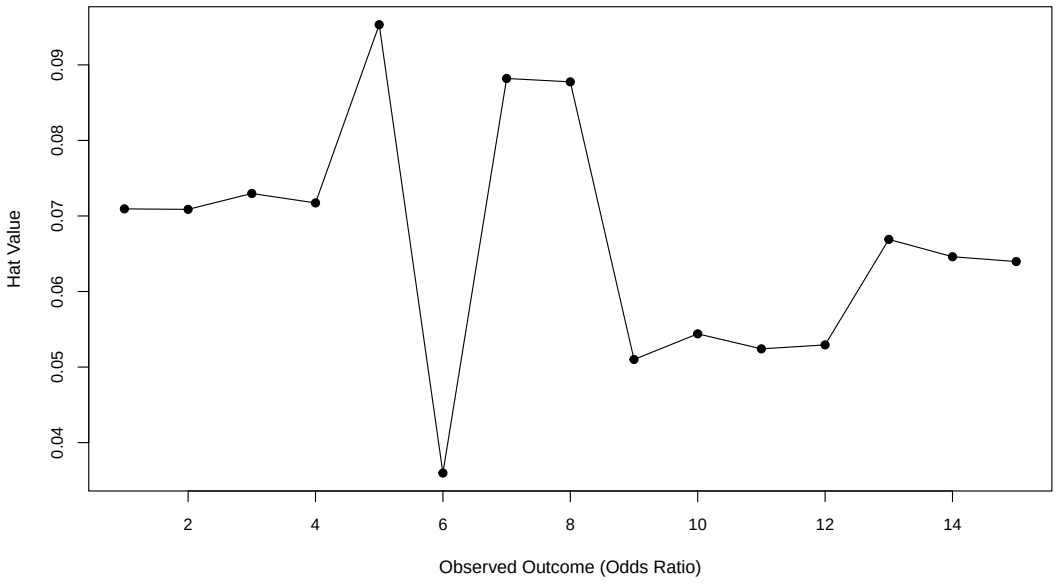


Figure 5: Hat Values plot assessing leverage of each of the 15 odds ratios.

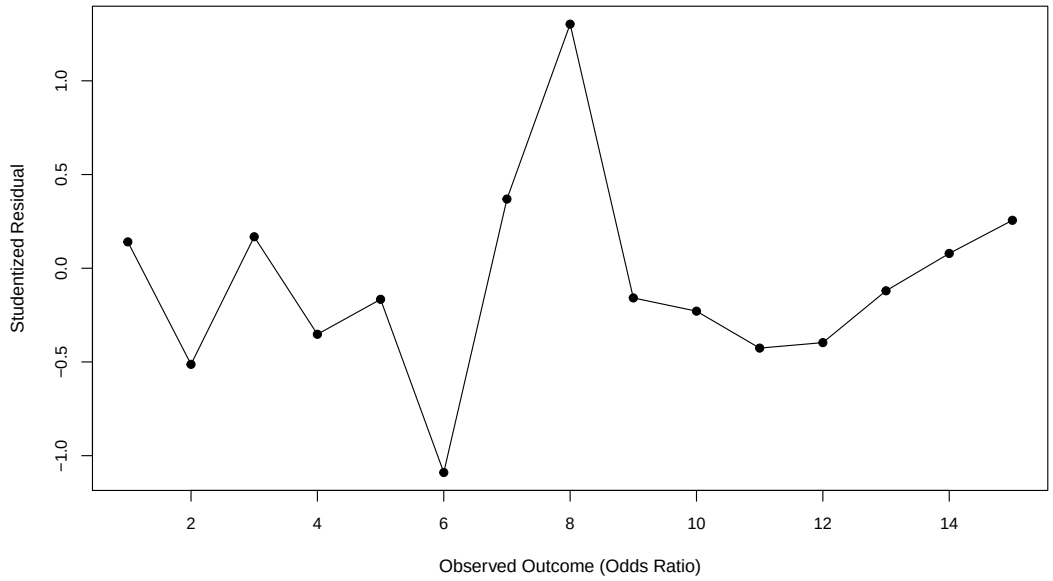


Figure 6: Studentized residuals plot assessing the error associated with each of the 15 odds ratios.

5.2.3 Results

The result of the final fitted model can be found in Table 4 and are summarized in the forest plot in Figure 7. The Odds Ratio for the association is greater than 1 which indicates that obese participants have higher

asthma prevalence than non-obese participants. However, the confidence interval slightly overlaps 1 which means that this is not a very strong result. This is further confirmed by the p-value of 0.066. This is relatively small but not small enough to confidently conclude a significant relationship between obesity and asthma prevalence overall.

Table 4: Final model results. Model with $\rho = 0.5$. Logged estimate and the odds ratio are reported.

Estimate	**Odds ratio**	**Standard error**	**p-value**	**CI (Odds ratio scale)**
0.3801	1.462	0.161	0.066	0.964, 2.219

Figure 7 is a forest plot which shows the association between obesity and asthma for each outcome in each study and the model estimate at the bottom. An odds ratio (OR) of one (red dotted line) indicates no effect and estimates with confidence intervals (horizontal lines) crossing one (vertical line) are more inconclusive. Powerful studies have narrower (shorter) confidence intervals. Associations with larger weights (less variation) have larger black squares. A study with an odds ratio of one and a very narrow confidence interval would indicate no significant effect. Here the Lu et al. (2016) effect size one, Barros et al. (2016) effect sizes, Jeong et al. (2017) effect sizes one and two and all of the Wang et al. (2015) effect sizes have 95% confidence intervals that do not cross one, indicating that these effect sizes would be judged statistically significant (at the 5% significance level). The pooled effect size (illustrated by a diamond) does cross one slightly, which is consistent with the model results in Table 4.

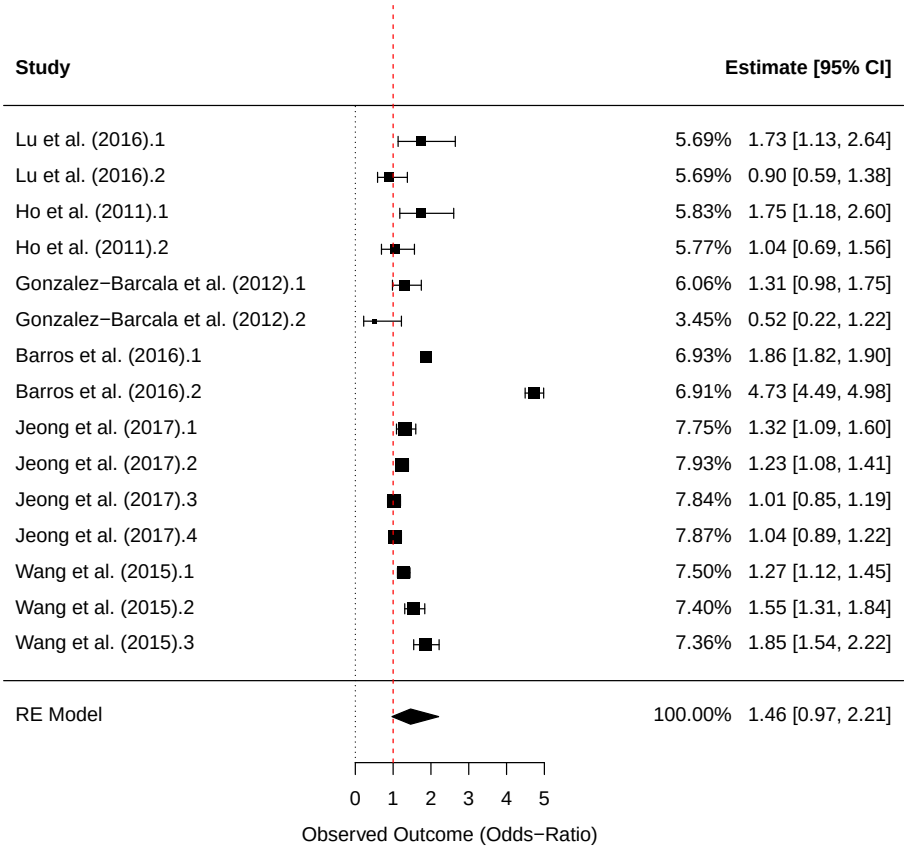


Figure 7: Forest plot of the association between obesity and asthma.

A radial plot is often used to supplement a forest plot, since it shows the same information, but in a different way. Figure 8 depicts the radial plot for all of the effect sizes from the included studies. The idea of a radial plot is to run an unweighted regression of z-scores (shown on the y-axis) on the inverse of the standard error (shown on the x-axis) with the intercept constrained to zero. These z-scores are calculated by taking each estimate and dividing it by its standard error. The curve on the right of the plot shows the actual value of the estimates. This plot is useful for determining outliers among the effect sizes and therefore, heterogeneity between observations/studies (if all of the studies are within the confidence interval lines (shaded area), then this would be an indication of no heterogeneity and outliers). The effect sizes from the Barros et al. (2016) study clearly lie outside the shaded area on the plot. This indicates that there is heterogeneity among the effect sizes and that these estimates are outliers. It has already previously been established that the outcomes from Barros et al. (2016) are outliers so this result is expected. The other effect sizes cannot be seen clearly in this plot, so the effect sizes from the Barros et al. (2016) study will be removed in the next plot to better visualize the other studies.

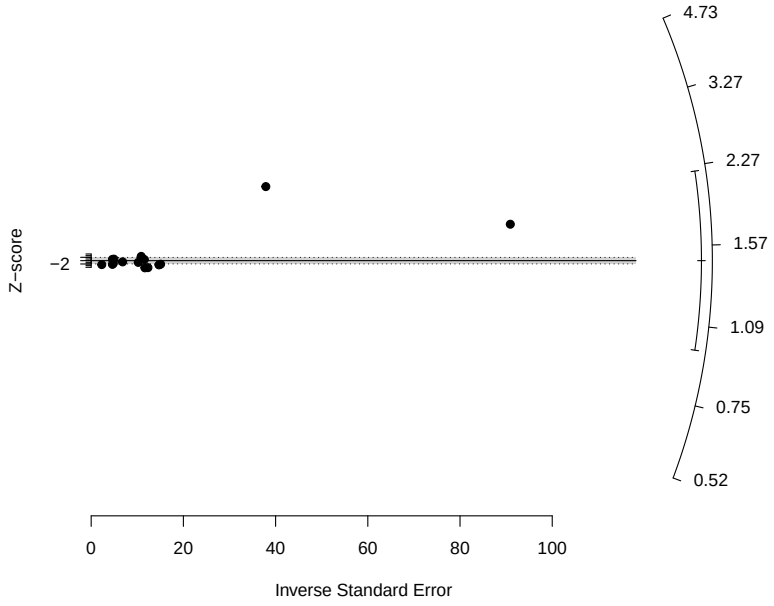


Figure 8: Radial plot of the association between obesity and asthma, including the effect sizes from the Barros et al. (2016) study.

Figure 9 shows consistency between most of the included effect sizes, since most of them lie within the shaded area. Five of the effect sizes lie outside of the shaded area which means that they could be outliers, but there is not enough evidence to warrant their removal from the included effect sizes. These studies are not too far outside of the shaded region so the threat of heterogeneity is not too high.

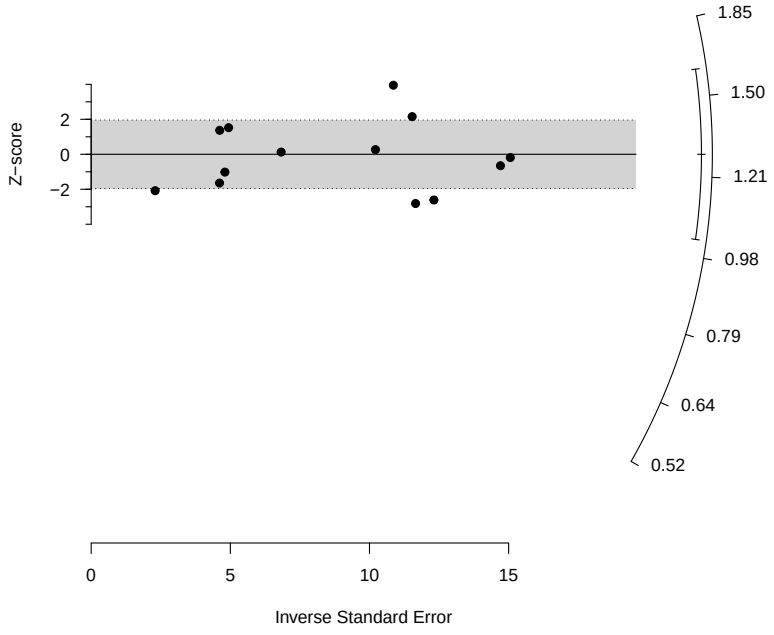


Figure 9: Radial plot of the association between obesity and asthma, excluding the effect sizes from the Barros et al. (2016) study.

Variance contributions are important to assess heterogeneity. The variance contributions of each level in the model can be seen in Table 5. The I^2 statistic describes the percentage of the variability in an effect estimate that is due to heterogeneity and not sampling error. It is calculated using:

$$I^2 = \frac{Q - df}{Q} \times 100$$

Where Q is the chi-squared statistic from a test for heterogeneity and df is the associated degrees of freedom.

The heterogeneity for level 2 (within-study variation) is slightly high. This is probably due to the fact that estimates from the same study tend to be quite different as they assess the relationship in different subcategories of the population. For example, the relationship between obesity and asthma prevalence seems to differ quite drastically between males and females and also between age groups. The heterogeneity in level 3 seems acceptable. Generally up to 50-60% would be categorized as only moderate heterogeneity and therefore not too much of a problem for combining estimates in the model. Barros et al. (2016) seems to be a substantial outlier and is probably responsible for increasing the heterogeneity between studies.

Table 5: Variance contributions (and I^2 statistics) of each level in the model.

Level	**% total variance**	$\boldsymbol{I^2}$
Level 1 - Between participants	1.53%	-
Level 2 - Between estimates	54.93%	54.93
Level 3 - Between studies	43.54%	43.54

5.3 Bayesian approach

5.3.1 Methods

Similiar to the frequentist meta-analysis method described in the section above, a Bayesian meta-analysis also pools observed effect sizes into one overall effect using a three-level mixed effect model. However, using a Bayesian model comes with some practical advantages over the conventional frequentist approaches, namely:

- Bayesian methods allow the uncertainty in our estimate of between-study and within-study heterogeneity to be modelled directly. Also, when the number of included studies is small, a Bayesian method is better at estimating pooled effects.
- Bayesian methods allow the full posterior distributions of the model parameters to be produced, which allows us to calculate the exact probability that any one of our parameters is larger or smaller than some specified value. This is in contrast to frequentist methods, which can only provide confidence intervals for model parameters and not probabilities of lying within a certain interval.
- Bayesian methods allow prior knowledge and assumptions to be integrated into the analysis. While in our example, we do not have strong prior assumptions, this can be useful in cases where there are.

Setting prior distributions:

Before the meta-analysis can be conducted, reasonable prior distributions need to be specified for our parameters, which have the same definitions as those defined for the frequentist three-level mixed-effect model. For our model, we decided on the following prior distributions:

$$\mu \sim \mathcal{N}(0, 1) \tag{6}$$
$$\zeta_{jk} \sim \mathcal{HC}(0, 0.5) \tag{7}$$
$$\zeta_{k} \sim \mathcal{HC}(0, 0.5) \tag{8}$$

For the true pooled effect size, μ, a Normal prior with a mean of 0 and variance of 1 was chosen, because it is reasonable for the true 95% probability of the pooled effect size (log-odds) to lie approximately between -2 and 2. Regarding the estimate of the variation between outcomes and between studies, ζ_{jk} and ζ_k, a Half-Cauchy distribution was chosen. Since the heterogeneity between outcomes and between studies cannot be negative, the location parameter was chosen to be 0. The distribution needs the majority of the density to fall between 0 and 1 as this is where the estimate of heterogeneity will most likely fall. However, it is best to include wider tails that extend considerably further than 1 to account for the possibility that the estimate is larger. Therefore, with regards to the scaling parameter, a conservative estimate of 0.5 was used. This flattens out the distribution and allows wider tails to allow for values further from 0 to still be relatively likely. A sensitivity analysis was also conducted to ensure that this choice of 0.5 for the scaling parameter does not have too great of an effect on the final estimate. This can be found in the statistical analysis section.

The model:

Now that the priors have been specified, the full model can be represented as:

$$\theta_{ijk} \sim \mathcal{N}(\theta_{ij}, \sigma^2_{ijk}) \tag{9}$$
$$\theta_{ij} \sim \mathcal{N}(\kappa_k, \zeta_{jk}) \tag{10}$$
$$\kappa_k \sim \mathcal{N}(\mu, \zeta_k) \tag{11}$$
$$\mu \sim \mathcal{N}(0, 1) \tag{12}$$
$$\zeta_{jk} \sim \mathcal{HC}(0, 0.5) \tag{13}$$
$$\zeta_{k} \sim \mathcal{HC}(0, 0.5) \tag{14}$$

where the terms are all as defined in the frequentist approach.

5.3.2 Statistical Analysis

Model Fitting

For the Bayesian model, a three level hierarchical model was fitted but using the priors defined above to generate estimates. The *brms* package was used to fit the model and generate the posterior distributions. The number of iterations used was 10000 with a warm-up of 5000 and 4 chains to achieve model convergence. A sensitivity analysis was also conducted to assess the change in the estimates when the scale parameter of the half-cauchy distribution is changed. The half-cauchy with scale parameter 0.5 is moderately restrictive while still having large tails to accommodate the possibility of larger heterogeneity vales. Decreasing this scale parameter value makes the distribution too restrictive and increasing it makes it more uninformative. Figure 10 demonstrates the change in the distribution for a few different values of the scale parameter. The results from the sensitivity analysis (shown in Table 6) indicate the 0.5 was probably a good choice. Any value from 0.3 to 1 results in very little change in any of the estimates. Very low values like 0.1 result in a relatively large decrease in the between-study heterogeneity. However, this has little effect on the overall estimate of the relationship between obesity and asthma. It is safer to overestimate heterogeneity than underestimate. This is why the scale parameter will be kept as 0.5, as a value below 0.3 results in too drastic a decrease in the between-study heterogeneity estimate.

Table 6: Results from a sensitivity analysis for the Bayesian hierarchical model for different values of the half-cauchy scale parameter.

Scale parameter	**Overall estimate**	**Between-study heterogeneity**	**Between-outcome heterogeneity**
0.1	0.37	0.17	0.39
0.3	0.35	0.27	0.37
0.5	0.35	0.31	0.38
0.7	0.36	0.32	0.38
1	0.36	0.33	0.39

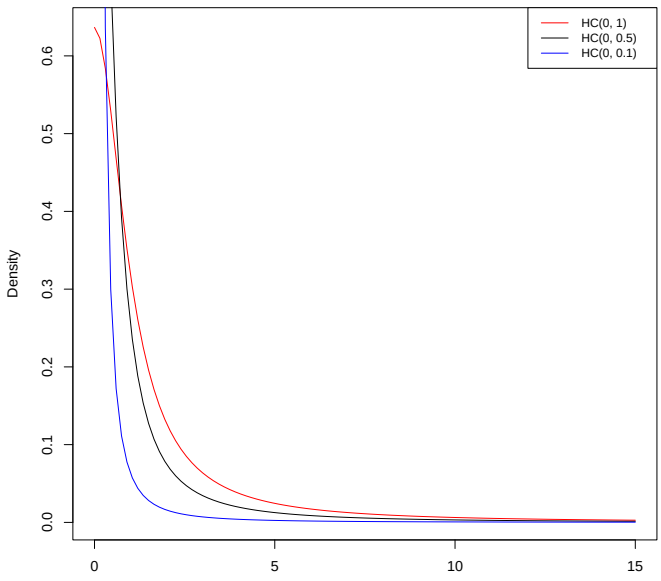


Figure 10: Possible Half-Cauchy prior distributions for different values of σ (the scale parameter).

Model Validation

Bayesian model validation involves checking for model convergence (that the Markov Chain Monte Carlo algorithm has found the optimal estimate). There are two ways to do this, the first being the $\hat{R}$ statistic (potential scale reduction factor). This is a way to check whether a chain has converged to the equilibrium distribution and it involves comparing the behavior of the chain to other randomly initialized chains (Harrer et al., 2021). A value below 1.01 indicates that the model has converged. The population-level, outcome-level and study-level estimates all had an $\hat{R}$ value of 1, indicating that they had converged. A posterior predictive check is another way to check model convergence. This involves randomly drawing data from the posterior distribution to simulate data and comparing this to the observed data. This is shown in Figure 11. The solid black line represents the observed data and the more transparent lines are the simulations. The simulations mostly replicate the distribution in the observed data well which indicates convergence.

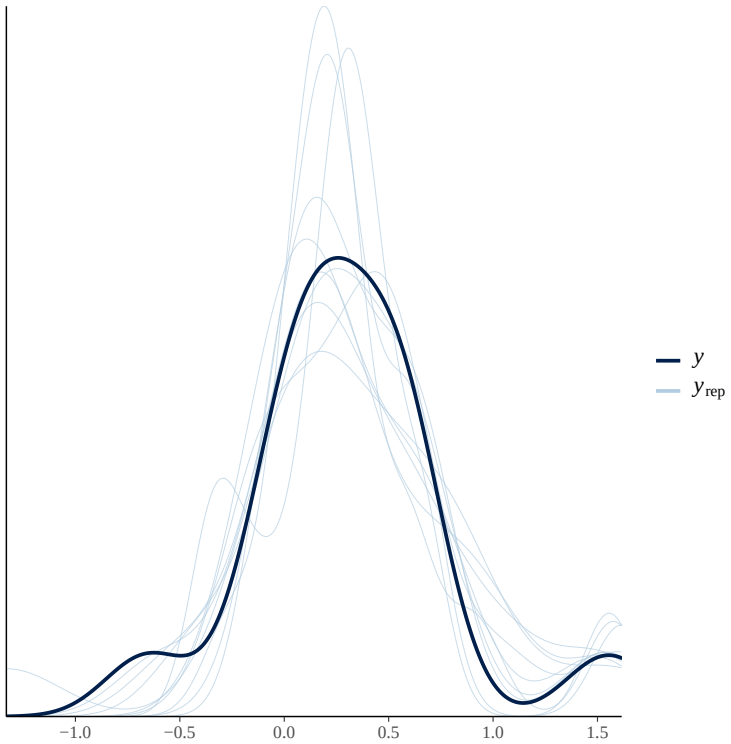


Figure 11: A plot of a posterior predictive check for the Bayesian model.

A leave-one-out sensitivity analysis was also conducted to assess the influence of individual outcomes in the model. The results of this analysis are illustrated in Figure 16 in the results section, below.

5.3.3 Results

The results for the Bayesian hierarchical model using:

$$\zeta_{jk} \sim \mathcal{HC}(0, 0.5)$$
$$\zeta_{k} \sim \mathcal{HC}(0, 0.5)$$

can be found in Table 7. The estimate is very similar to the frequentist approach. However, the credible interval is narrower than the confidence interval from the frequentist approach. This interval is interpreted as the probability that the estimate lies between 0.97 and 2.03 is 0.95.

The estimate of the between-study heterogeneity in the model is shown in Table 8. The deviation of each study estimate from the population-level estimate is shown in Table 9. Most studies except for Barros et al. (2016) and Wang et al. (2015) have a negative deviation from the population-level estimate. All studies also have a relatively low deviation except for Barros et al. (2016). The within-study heterogeneity (also shown in Table 8) is slightly larger than the between-study heterogeneity. This indicates that different population groups within a study differ more from each other that the studies do. This is consistent with the results from the frequentist approach which estimates the between-outcome variance contribution to be higher than the between-study contribution.

Table 7: Final Bayesian model results. Logged estimate and the Odds Ratio are reported.

Estimate	**Odds ratio**	**Standard error**	**Credibility interval (Odds ratio scale)**
0.35	1.42	0.19	0.97, 2.03

Table 8: Estimates of within-study and between study heterogeneity.

Source	**Estimate**	**Standard error**	**Credibility interval (Odds ratio scale)**
Within-study	0.38	0.11	0.22, 0.62
Between-study	0.31	0.19	0.02, 0.79

Table 9: Estimated deviation of each study estimate from the overall effect size.

Study	**Estimate**	**Error estimate**	**Credible interval**
Barros et al. (2016)	0.378	0.296	-0.077, 0.993
Gonzalez-Barcala et al. (2012)	-0.158	0.250	-0.723, 0.284
Ho et al. (2011)	-0.024	0.224	-0.499, 0.424
Jeong et al. (2017)	-0.134	0.211	-0.584, 0.266
Lu et al. (2016)	-0.060	0.228	-0.550, 0.393
Wang et al. (2015)	0.044	0.207	-0.367, 0.491

The forest plot displaying the estimates for each study and for the overall population-level odds ratio is shown in Figure 12. The posterior distributions are also shown for all estimates. Most of the posterior distributions are roughly normal with some being flattened or skewed slightly. Some also peak higher than others. The population-level distribution is quite flat and stretched, probably to include all of the heterogeneity in the estimates. Figure 13 shows the same forest plot but for the study-level estimates and not the outcome-level. The study level distributions all look normally distributed. Barros et al. (2016) has a very rounded posterior distribution compared to the other studies.

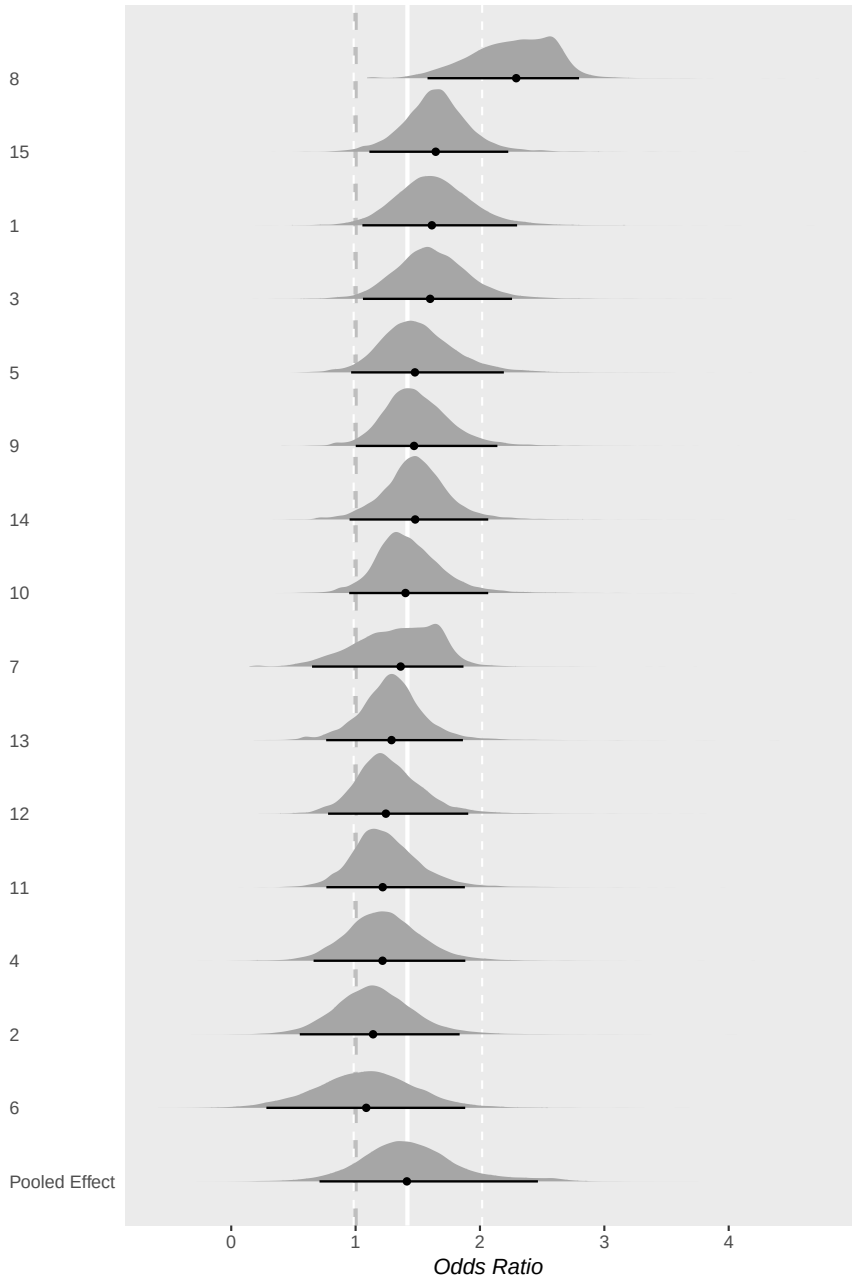


Figure 12: Forest plot with the distribution for each OR and the overall OR. The black dot represents the estimate and the black lines show the interval in which 95% of the density lies.

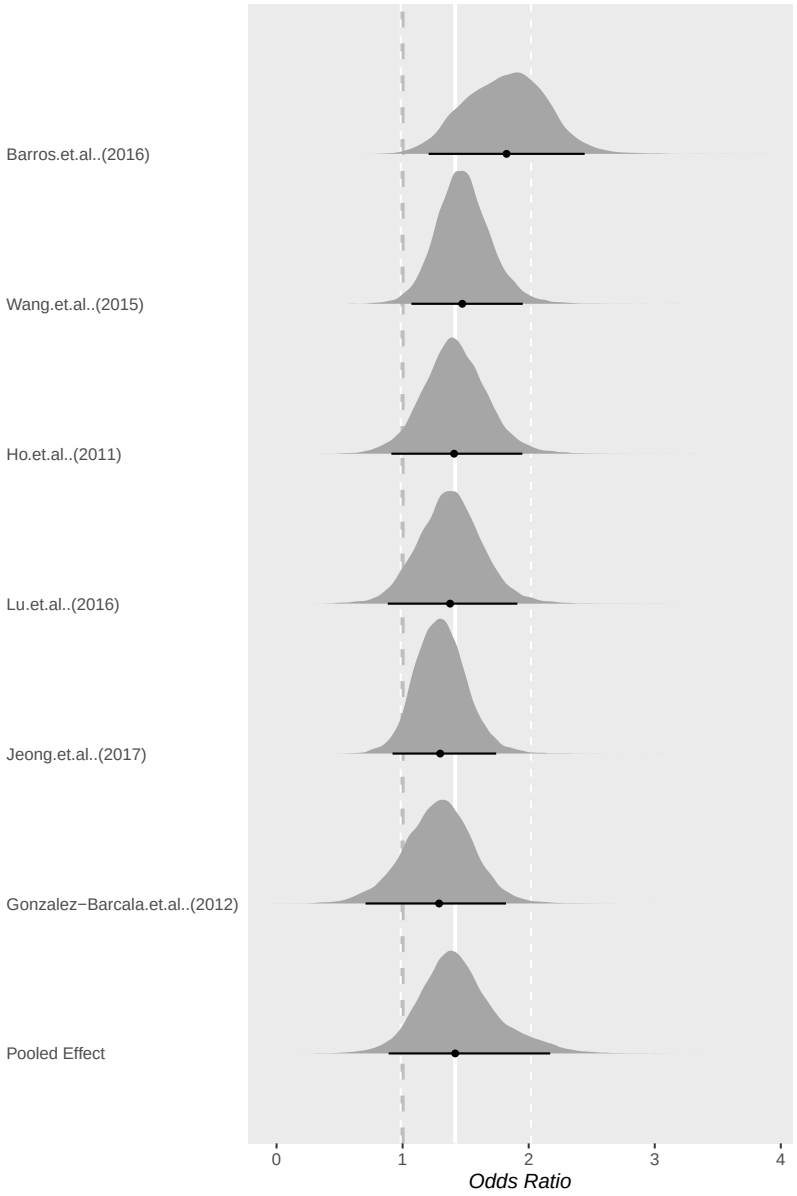
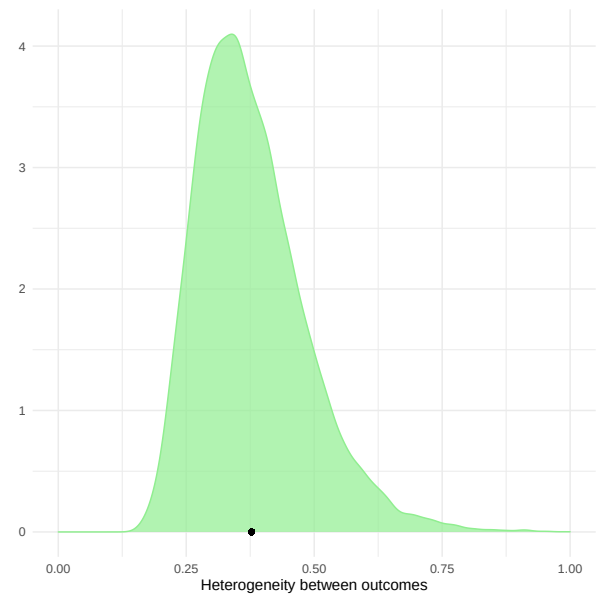
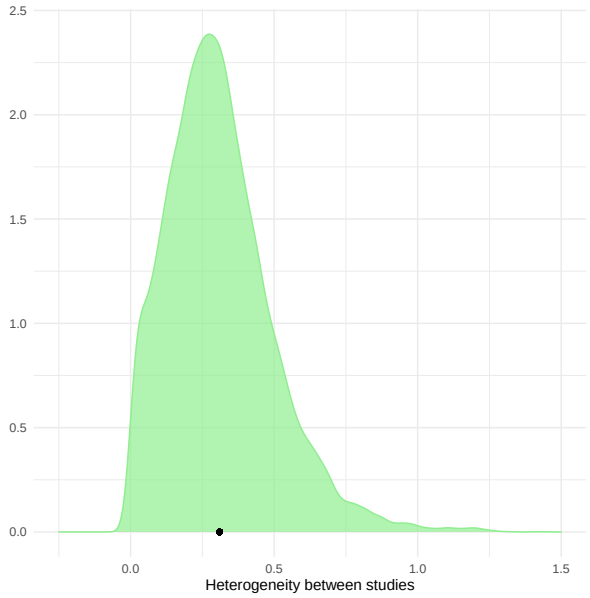


Figure 13: Forest plot with the distribution for each study OR and the overall OR. The black dot represents the estimate and the black lines show the interval in which 95% of the density lies.

Figure 14 shows the posterior distributions of the within-study (between-outcome) heterogeneity in sub-figure 14a and between-study heterogeneity in sub-figure 14b. These estimates are both positive and the distributions barely overlap 0. This is expected as the estimates of heterogeneity should be restricted to be positive (this was the purpose of the half-cauchy prior). Both heterogeneity posteriors resemble skewed normal distributions which are skewed slightly to the right. Again, this makes sense as the distributions should be positive so the right skew allows more variability on the positive side and less on the negative. The posterior distribution of the pooled effect (odds ratio) is shown in Figure 15. This distribution is also normal and is quite symmetric around the estimate, except for the bump on the left-hand side of the distribution. This bump is possibly caused by an outlying estimate, possibly outcome 6 (This is the estimate for 13-14 year olds from Gonzalez-Barcala et al. (2012), which actually showed a negative association between obesity and asthma).



(a) Posterior distribution of the heterogeneity between outcomes.



(b) Posterior distribution of the heterogeneity between studies.

Figure 14: Heterogeneity posterior distributions.

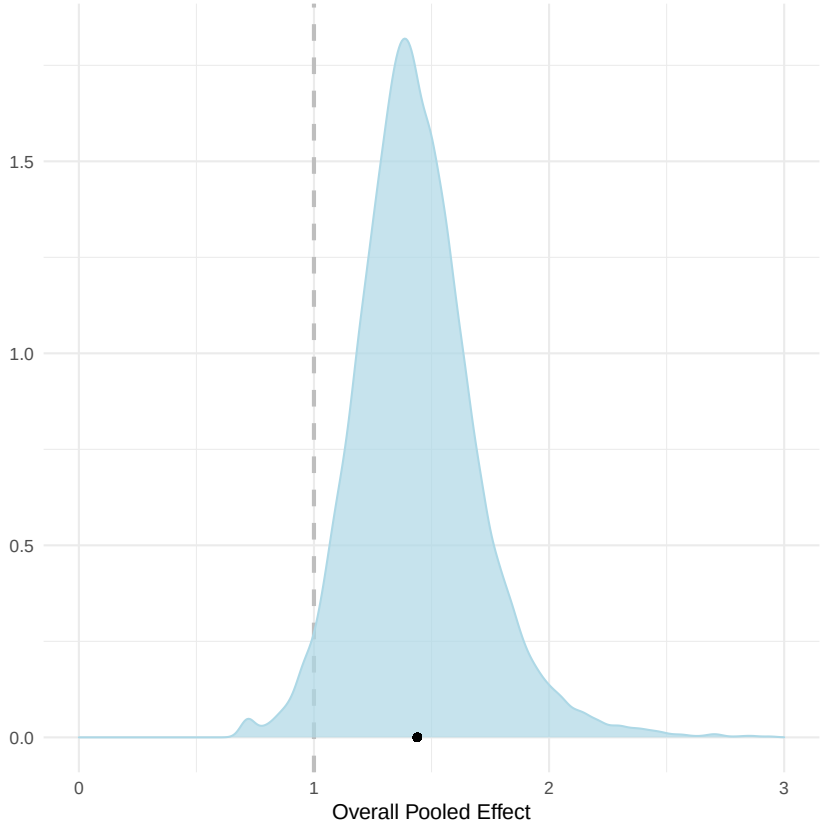


Figure 15: Posterior distribution for the overall estimate (odds ratio).

The results from the leave-one-out sensitivity analysis can be found in Figure 16. The estimate and 95% credibility interval does not change much when individual outcomes are removed from the analysis. Outcomes 7 and 8 (from Barros et al. (2016)) have the highest influence on the estimate. This was also the case in the frequentist model. These two estimates also seem to have a large effect on the posterior distribution as the shape of the distribution changes quite drastically when they are removed.

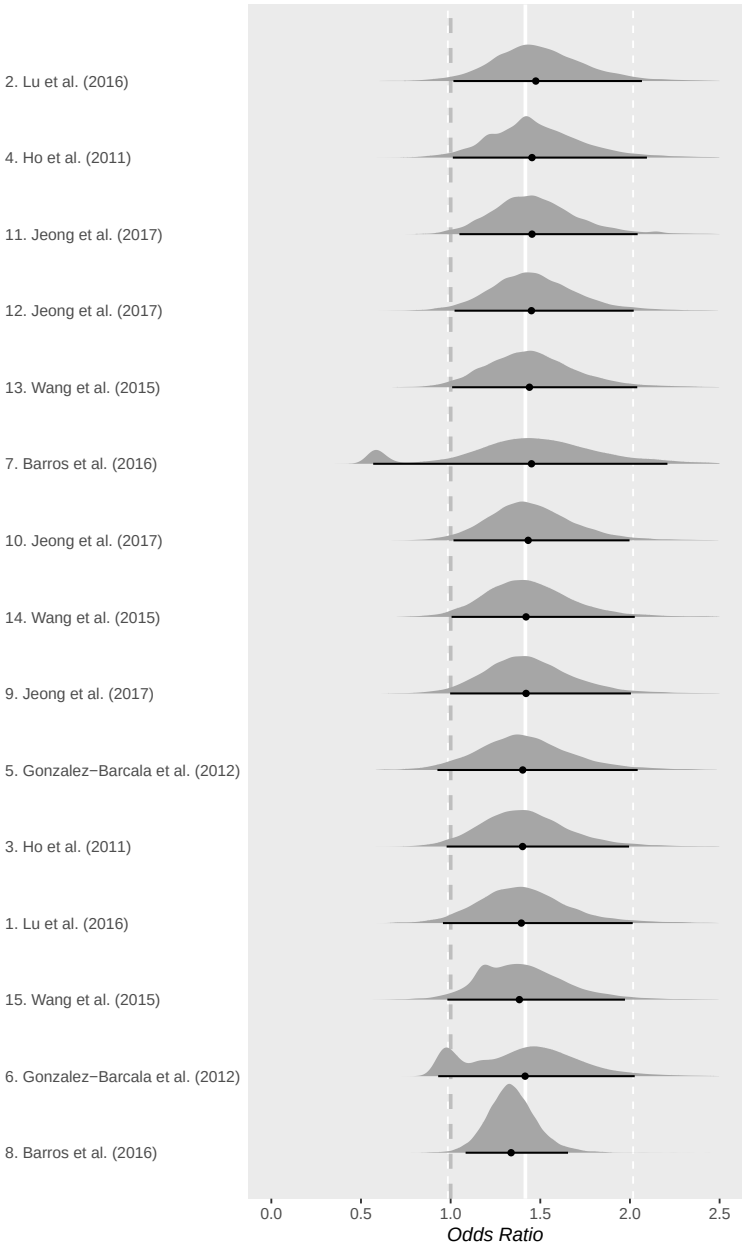


Figure 16: A plot of the results from the leave-one-out sensitivity analysis. The labels on the left indicate the estimate left out in each model.

5.4 Publication bias

Publication bias can be an problem when conducting a systematic review and meta-analysis, especially when only published studies are used in the review. Publication bias refers to the tendency of studies with

favourable results to be published over studies with non-favourable results (Higgins et al., 2022). It occurs when the choice to publish or speed at which a study is published depends on the magnitude and direction of the study's effect estimates (Higgins et al., 2022).

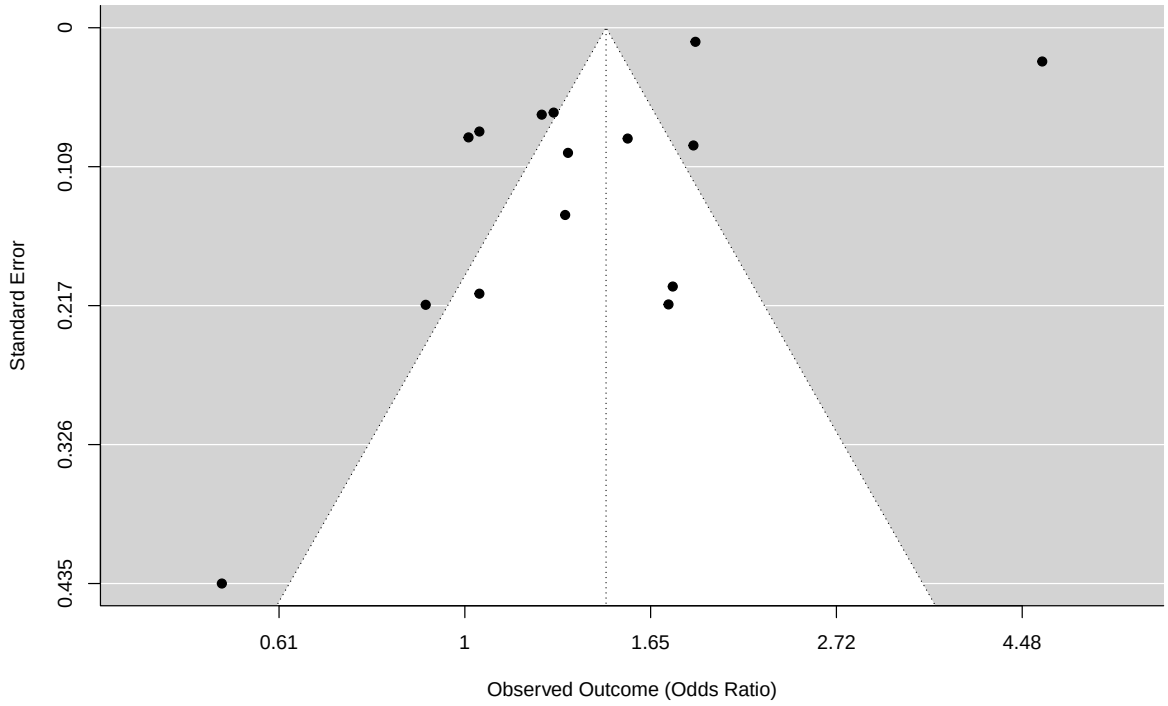


Figure 17: Funnel plot indicating the risk of publication bias in the included studies.

Above in Figure 17 is a funnel plot that shows some possible publication bias. Each dot represents an effect size; the y-axis represents study precision (the standard error) and the x-axis shows the study's result (as an odds ratio). The standard error was used on the y-axis, since straight lines can be drawn to define a region within which 95% of points might lie in the absence of both heterogeneity and publication bias. The vertical line in the middle of the funnel shows the average effect size across all of the studies (from the frequentist approach). A funnel plot assumes that estimates with low precision will vary more from the average but should be spread evenly on either side of the average (Sterne et al., 2011). For high precision estimates, it assumes that they should be near the average (Sterne et al., 2011). This is because, in the absence of heterogeneity and publication bias, estimates with high precision (which usually means a large sample size too) are expected to vary less than studies with lower precision (which usually means a smaller sample size too) (Sterne et al., 2011). The funnel plot shown in Figure 17 indicates some possible problems. While it can be seen that studies with lower standard errors lie more concentrated around the estimated true effect, the pattern overall looks asymmetrical. This is because there is one study with a low effect size in the bottom-left corner of the plot and the two large effect sizes from the Barros et al. (2016) study in the top-right corner of the plot. These effect sizes, however, have no equivalent in the bottom-right or top-left corners of the plot. Another troubling detail is that the study with the greatest precision in our sample, the one by Barros et al. (2016), does not seem to follow the funnel pattern well either. Its effect size is considerably larger than expected. However, it is important to note that it has already been established that there is some heterogeneity in the effect estimates (Odds ratios). This heterogeneity also affects the funnel plot and does not necessarily indicate publication bias.

The exposure and outcome assessed here (obesity and asthma) also does not indicate an obvious reason for publication bias. If the exposure is a treatment or drug, there tends to be more incentive to publish favourable results as there is usually large monetary investment involved (Sterne et al., 2011). Most of the studies included in this review are smaller, non-funded research papers.

5.5 A Comparison of the Paradigms

Both the frequentist and Bayesian paradigms produced remarkably similar results, in terms of the overall estimates and the amount of heterogeneity accounted for between and within-studies. There is no overarching reason why one approach would be recommended over the other and the choice of which to use would be mostly based on preference. However, each approach does have its own benefits. The frequentist approach is more widely used for meta-analyses which means that the packages and implementation are much more user-friendly. This approach would be recommended for anyone who does not have a good base knowledge of statistics. The Bayesian approach also requires more computational power, so if this is a consideration the frequentist approach may be the better choice. The main benefits of the Bayesian approach is the ability to incorporate prior knowledge, the direct modelling of the heterogeneity and it's ability to handle data with more heterogeneity than the frequentist approach. Especially when the number of studies included in the meta-analysis is low, the Bayesian approach is likely to produce a more accurate estimate of the association between an exposure and outcome. Ultimately, the choice of which paradigm to implement in a meta-analysis will come down to the individual implementation and which method is most suited or preferred for that implementation. However, the Bayesian approach is recommended when the number of studies included in the meta-analysis is very low.

6 Mendelian randomisation

6.1 Introduction

6.1.1 The problem

Biomedical researchers seek to understand the causes of ill health so that informed decisions can be made, which are subsequently beneficial to the public. This requires the identification of causal associations between an exposure and an outcome (Harrison, Howe & Davies, 2020). However, to analyse these associations successfully, two key problems need to be addressed first: confounding and reverse causation.

Confounding variables are measurable factors that affect the exposure-outcome relationship; these can be adjusted for in a statistical analysis. However, even if a variable is a known confounder, it is unlikely that it has been measured perfectly, especially for complex problems such as socioeconomic circumstances (Harrison, Howe & Davies, 2020). Hence, controlling for this is less effective. There can also be unknown confounders which have not been measured or considered by people investigating an association. This type of confounding is known as residual confounding and is present in nearly all observational studies. For example, an observational study may find that obesity is associated with an increased odds of late-onset asthma. However, this conclusion may be incorrect if other factors, such as hypertension, are responsible for the perceived relationship between the exposure and outcome (Harrison, Howe & Davies, 2020).

Reverse causality is defined as an incorrect assignment of the direction of an association between two variables (Harrison, Howe & Davies, 2020). Referring to the example above, this would be akin to claiming that asthma causes obesity when the actual relationship is the opposite.

6.1.2 The traditional approach to determining causal relationships

The standard method to address both confounding and reverse causality is a randomised controlled trial (RCT). A RCT randomly splits a sample of participants into two or more groups, where one group receives the intervention and the other group(s) receive a control or different intervention. Due to the random assignment of participants to treatment groups, the only difference between the groups should be whether or not they received the intervention. This means that there should be no residual confounding, as groups are equivalent. There also cannot be reverse causality, because the intervention comes before the outcome of interest. The issue we face in these conditions is that for many research questions, randomly assigning the exposure is impractical or unethical. For example, it would not be possible or acceptable to allocate obesity randomly to participants (Harrison, Howe & Davies, 2020). In these cases, Mendelian randomisation may be useful.

6.1.3 Mendelian randomisation

Mendelian randomisation is a process for determining causality between two traits using observational data. It involves using SNPs (Single Nucleotide Polymorphisms) that are associated with an exposure and the outcome (through the exposure) to determine whether the exposure causes the outcome (Burgess et al., 2020). A SNP is a single nucleotide position in the genome where the allele present in that position can vary between individuals in a population (Harrison, Howe & Davies, 2020). An allele refers to a variation of the same piece of DNA (a single nucleotide in this case). SNPs are often associated with a trait and this is what is exploited to conduct a Mendelian randomisation study (Harrison, Howe & Davies, 2020). SNPs are used as a proxy for the exposure and the relationship between these SNPs and the outcome is assessed. Using genetic markers as a proxy for a trait removes the possibility that confounding variables can affect the exposure or the relationship between the exposure and outcome. This is because confounders should not influence an individual's genetic predisposition towards the exposure (Burgess et al., 2020). This is illustrated in Figure 18 below.

A Mendelian randomisation investigation can be conducted using individual level data or summary statistics. Using individual level data can be very time consuming and costly as it involves genotyping all participants.

Due to this, it is more common for Mendelian randomisation studies to be conducted using summary statistics from published GWAS (Genome Wide Association Study) results (Burgess et al., 2020).

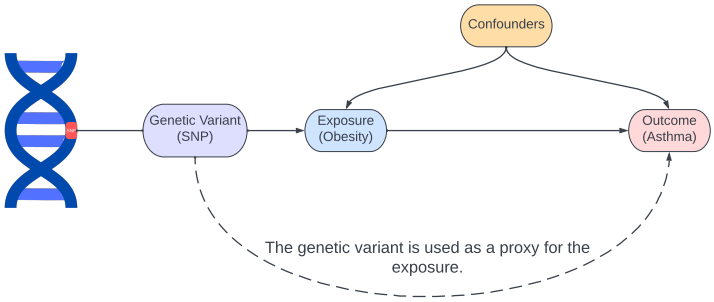


Figure 18: A diagram depicting the use of genetic variants to assess the causal association between obesity and asthma. By using genetic variants as a proxy for the exposure, the effect of confounders is mitigated.

Instrumental variables (IV) and Mendelian randomisation assumptions:

A key assumption is that the genetic variants used in a Mendelian randomisation study must be associated with the exposure variable. This assumption can be verified by testing the association between the genetic variants and the exposure variable in the data or by collecting summarised data from published GWAS studies where the association has already been assessed (Harrison, Howe & Davies, 2020).

A second assumption is that the genetic variants must not be directly associated with the outcome variable or other biological pathways that could possibly affect the outcome. Biological knowledge about the genetic variants can determine how likely meeting or violating this assumption is. When a variant is directly associated with the outcome or other pathways outside of the association of interest this is known as horizontal pleiotropy (see Figure 19) (Harrison, Howe & Davies, 2020). This can create the illusion that the exposure is associated with the outcome (or associated with the outcome more strongly than it actually is). Vertical pleiotropy occurs when a SNP is associated with a trait through an association with another trait (see Figure 19). This vertical pleiotropy between the exposure and outcome variables is what we are trying to detect in a Mendelian randomisation investigation. Specifically, our goal is to find SNPs that are associated with obesity as well as asthma through their association with obesity. The difference between horizontal pleiotropy and vertical pleiotropy is illustrated in Figure 19. For the rest of this section, when pleiotropy is referred to, this is referring to horizontal pleiotropy.

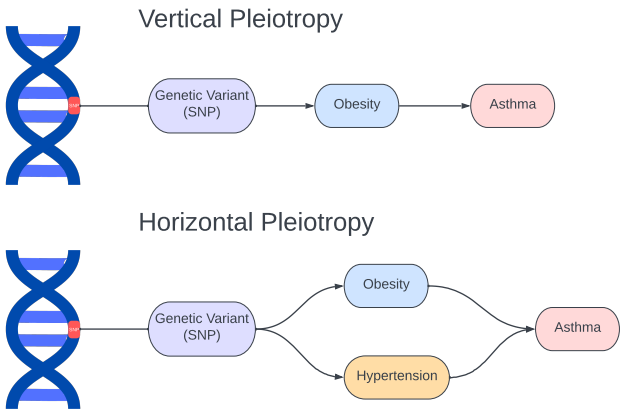


Figure 19: A diagram depicting vertical vs horizontal pleiotropy. Here, obesity is the exposure, hypertension is a confounding variable and asthma is the outcome.

The third assumption is that there is no linkage disequilibrium between any of the identified SNPs. Linkage disequilibrium occurs when the association of alleles at different loci is not random (Burgess et al., 2020). This means that two or more SNPs can be observed to be inherited together more or less often than what would be expected from random association. This would mean that a SNP can be associated with the exposure and possibly also the outcome, not because it affects the exposure, but because it is linked to another SNP that affects the exposure. This can lead to an inflated estimate of the effect of the exposure on the outcome. A comparison of linkage equilibrium and linkage disequilibrium is illustrated in Figure 20 below. This shows an example of how the allele frequencies can differ when two SNPs are in linkage disequilibrium.

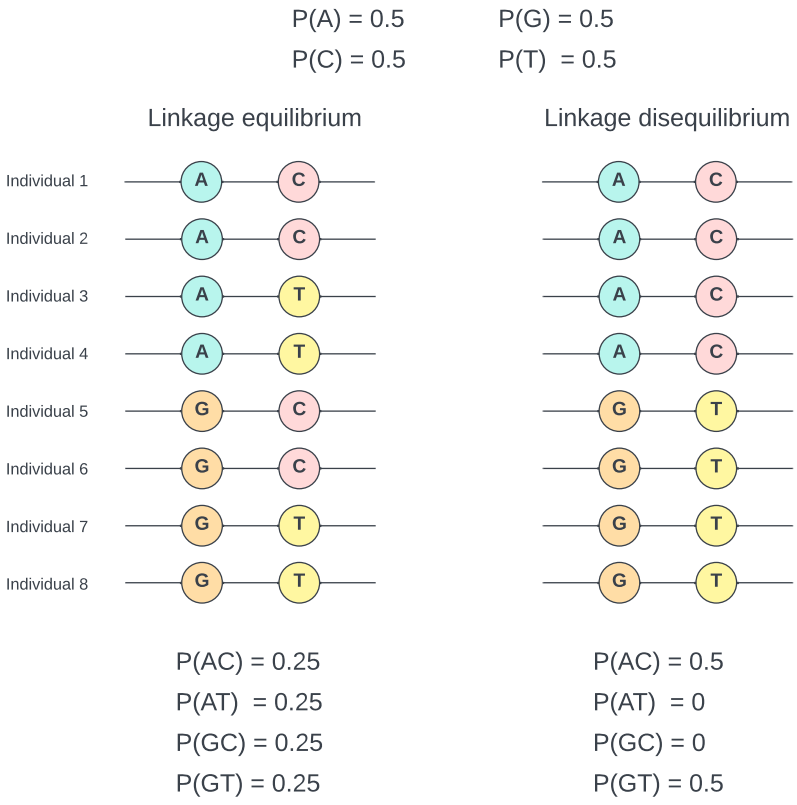


Figure 20: A diagram depicting linkage equilibrium vs linkage disequilibrium. This shows the same two SNPs in 8 different individuals. On the left are the frequencies of the combination of the two SNPs one would expect if these two SNPs were in linkage equilibrium. Displayed on the right are frequencies of the combination of these two SNPs in linkage disequilibrium.

The assumptions mentioned above form the definition of an instrumental variable. A variant satisfying these assumptions divides a study population into subgroups analogous to treatment groups in a randomised controlled trial in that they differ systematically concerning the exposure of interest, but not with respect to confounding factors. Therefore, an association between the genetic variant and the outcome implies that the exposure has a causal effect on the outcome (Burgess et al., 2020). Also, if these instrumental variable assumptions are met, then reverse causation is not a problem. This is because the SNP is not directly associated with the outcome variable, which implies that the outcome variable is not the causal factor. If these assumptions are not met, the results of the Mendelian randomisation analysis can be biased. This could mean that the strength of the causal association is overestimated or that a causal association is reported when, in reality, there is not one.

6.1.4 Limitations of Mendelian randomisation

Mendelian randomisation is limited to exposures which have a genetic basis. For example, it cannot be used to determine whether living in a city causes asthma since no genetic variants are likely to predispose people to live in a city.

Another limitation is that there may not be enough research into the relationships between genetic variants directly linked to an exposure of interest (e.g. the biological functions of the variants are not well understood) (Harrison, Howe & Davies, 2020). Thus, making the instrumental variable assumptions difficult to validate from a biological standpoint.

Given the small effect sizes of genetic variants, large sample sizes are needed to determine an effect accurately. Extensive studies such as the UK Biobank have made the collection of data more accessible. However, the populations recruited for these studies often do not represent the wider population, which can introduce selection bias into the results. This risk can be reduced by encouraging diversity in population cohorts and developing statistical approaches to account for selection bias (Harrison, Howe & Davies, 2020).

6.2 Data preparation

6.2.1 Specification of the datasets

A Mendelian randomisation study using published data can be conducted using a single-sample or two-sample approach. The single-sample approach involves using summary data from a single study which summarises genetic associations with the exposure and the outcome of interest (Burgess et al., 2015). A problem with this approach is that it is uncommon to find a study which includes summarised data on the associations between the exposure and outcome of interest. It also means that the sample for the exposure associations and outcome associations are the same (and are, therefore, not independent) which has to be accounted for in the analysis.

Two-sample Mendelian randomisation is an approach that attempts to rectify this problem by collecting data on the associations between the genetic variants and the exposure and outcome from non-overlapping data sources. An important assumption made to ensure the validity of the analysis is that the two sets of samples come from the same underlying population. This is because the genetic make-up of individuals from the same population is more similar than between individuals from different populations. So the associations with the exposure and outcome may not be replicated in individuals from different populations. The instrumental variable assumptions may also not hold outside of the population in which they were made. (Burgess et al., 2015).

Using published summary data rather than individual-level data has may advantages, most importantly, the size and scope of the studies. This allows for more precise estimates of the associations between variants and the exposure and outcome variables. Genetic association data can also be a difficult form of data to generate as it involves genotyping all participants. This makes it much easier and more efficient to use summary data from big genome wide association studies.

For this analysis, two datasets were used, obtained from Locke et al. (2015) and Valette et al. (2021). The study by Locke et al. (2015) set out to find genetic variants associated with obesity (BMI $\geq$ 30) and found 77 significant BMI-linked SNPs. These SNPs were from GWAS and Metabochip studies (n = 322,154) and achieved genome-wide significance (GWS) (Locke et al., 2015). The study by Valette et al. (2021) primarily tried to identify genetic variants causally associated with asthma using data from the UK Biobank using a broad definition of asthma (n = 56,167 asthma cases and 352,255 controls). The study found 72 asthma-associated loci from 116 independent significant variants ($P_{GWAS} < 5.0 \times 10^{-8}$). More importantly for our analysis, the study's association data also included summaries of the associations between the 77 genetic variants from the study by Locke et al. (2015) and asthma. Locke et al. (2015) made use of the GIANT consortium which consists of participants of European-descent and Valette et al. (2021) made use of the UK Biobank consortium which consists of participants of British descent. While ideally both datasets should be created using European participants from numerous countries, the UK is within Europe. Hence, participants should not differ too drastically from a genetic perspective.

6.2.2 The search for candidate instrumental variables

As mentioned above, the study by Locke et al. (2015) identified 77 significant BMI-associated genetic variants. These genetic variants were selected as our candidate instrumental variables. The gene-outcome associations were obtained from the study by Valette et al. (2021). If the gene-exposure or gene-outcome association was not available, a proxy for that variant was used. A proxy is a variant that is in complete or near complete linkage disequilibrium with the original variant. To be in complete linkage disequilibrium it means that these SNPs are almost always inherited together so the association of the proxy with the outcome can be assessed instead of the original SNP. This was the case with one SNP in our dataset which was replaced by a proxy.

Since two-sample Mendelian randomisation was used and the datasets used did not overlap, any bias from instrumental variables not strongly associated with the risk factor (weak instruments) is in the direction of the null. This means that using a large number of genetic variants which are valid instrumental variables should not result in false positive causal claims.

6.2.3 Validation of the instrumental variable assumptions

The instrumental variable assumptions for a set of variants are vitally important to a Mendelian randomisation study. However, the assumptions are not all empirically testable, which means that they cannot be completely verified and need to be justified by biological understanding as well as statistical testing. The three instrumental variable (IV) assumptions that need to be validated are (Burgess et al., 2020):

1. The variant must be associated with the exposure / risk factor of interest.
2. The variant must be independent of confounders of the risk exposure–outcome association.
3. The variant can only affect the outcome through the exposure. A change in the value of the genetic variant that does not have an effect on the exposure must also not affect the outcome.
4. The variant should not be in linkage disequilibrium with any other variant included in the analysis.

With regard to biological understanding, if the function of the gene in which the variant is located is known, this may give a clue as to whether the variant is a plausible instrumental variable. However, assessing the biological function is beyond the scope of this study and was not done here.

With regard to statistical testing, an effective method of assessing the instrumental variable assumptions is by testing the association of the candidate genetic variants with a range of covariates which are potential confounders. This would again require knowledge about the SNPs of interest beyond the scope of this study and was also not done here. While this was not controlled for while selecting instrumental variables, an MR-Egger regression model was fitted (more information on this model can be found in the methods sections) which accounts for possible pleiotropy. A test for horizontal pleiotropy was also conducted after the analysis. Linkage disequilibrium between the 77 selected SNPs was tested using the *LDlinkR* package in R. This indicated no linkage disequilibrium and therefore, all 77 SNPs were included in the analysis.

6.2.4 Variant harmonisation

The BMI and Asthma estimates were combined into a single data set and harmonisation was performed. This ensures that the effect of a SNP on the exposure and the effect of that SNP on the outcome correspond to the same allele. Most GWAS studies report effects in reference to the allele on the forward strand of DNA but it is safer to harmonise anyway as some older studies do not use this convention and there can be some discrepancies. This resulted in one SNP being removed (rs1558902). This is because this SNP is palindromic, so the alleles on the forward strand are the same as the alleles on the reverse strand (for example: G/C on forward strand and C/G on the reverse strand). This SNP also has a minor allele frequency above 0.42. This means that allele frequency cannot be used to infer which strand the effect allele is on.

Approaches

Mendelian randomisation, like a meta-analysis, can be conducted using a frequentist or Bayesian approach. Both have been implemented here. The data preparation for both approaches is the same. The frequentist approach is presented first, followed by the Bayesian approach.

6.3 Frequentist Approach

6.3.1 Methods

For each of the, now, 76 genetic variants the estimate of the genetic association with the risk factor is represented as X_k with standard error σ_{Xk} and the estimate of the genetic association with the outcome as Y_k with standard error σ_{Yk}. The genetic associations are usually per allele effects: the change in the exposure or outcome for each additional copy of the minor (or effect) allele, this is the case for our association with the exposure (X_k). The outcome of asthma onset is binary, so Y_k is the regression coefficient from a logistic regression, represented as a log-odds ratio.

The two methods used in our analysis for the estimation of a causal effect were an inverse-variance weighted (IVW) method and Mendelian randomisation Egger regression (MR-Egger) method. When the genetic associations with the risk factor are precisely estimated, both approaches give similar estimates (Burgess et al., 2020). On the other hand, when there is considerable imprecision in the estimates, causal effect estimates from the inverse-variance weighted method are over-precise. The MR-Egger method adjusts for this and gives appropriately-sized confidence intervals (Burgess et al., 2020).

The inverse-variance weighted (IVW) method:

When dealing with multiple uncorrelated genetic variants, the IVW estimate can be obtained from an IVW meta-analysis of the ratio estimates for the individual variants: The ratio estimates from each genetic variant can be averaged using an inverse-variance weighted formula taken from the meta-analysis literature to provide an overall causal estimate known as the inverse-variance weighted (IVW) estimate. This assumes that the ratio estimates all provide independent evidence on the causal effect (Burgess et al., 2020).

The linear model with no intercept, from which the IVW causal estimate is derived is:

$$Y_k = \beta X_k + \epsilon_k; \;\; \epsilon_k \sim \mathcal{N}(0, \sigma^2_{Y_k}) \tag{15}$$

Where the variables are as defined above and ϵ_k is the residual. The causal estimate from the IVW method is:

$$\hat{\beta}_{IVW} = \frac{\sum_{k=1}^{K} X_k Y_k \sigma_{Yk}^{-2}}{\sum_{k=1}^{K} X_k^2 \sigma_{Yk}^{-2}} \tag{16}$$

The approximate standard error of the estimate is:

$$se_{\hat{\beta}_{IVW}} = \sqrt{\frac{1}{\sum_{k=1}^{K} X_k^2 \sigma_{Yk}^{-2}}} \tag{17}$$

In the IVW method, the relationship between the exposure and the outcome is assumed to be linear and the estimate β_{IVW} expresses the causal increase in the log-odds of the outcome per unit change in the exposure.

The Egger regression (MR-Egger) method:

The MR-Egger method estimates the causal effect as the slope from the weighted regression of the variant-outcome associations on the variant-exposure associations, and the average pleiotropic effect as the intercept (Burgess & Thompson, 2017). This method allows all genetic variants to have pleiotropic effects, but it

requires that these effects are independent of the variant-exposure association (referred to as the Instrument Strength Independent of Direct Effect (InSIDE) assumption). Estimates from the MR-Egger method are particularly affected by outlying and influential data points. When the associations between the variants and exposure are all similar in magnitude, MR-Egger estimates are prone to imprecision. This results is wide confidence intervals which results in low power to detect causal effect.(Burgess & Thompson, 2017). The model below is based on work by Burgess & Thompson (2017).

The causal estimate from the MR-Egger method is:

$$Y_k = \beta_{0E} + \beta_{1E} X_k + \epsilon_{E_k}; \;\; \epsilon_{E_k} \sim \mathcal{N}(0, \sigma'^2 \sigma^2_{Y_k}) \tag{18}$$

where β_{0E} is the intercept, β_{1E} is the slope (MR-Egger estimate), σ'^2 is estimated residual standard error and ϵ_{E_k} is the residual.

The approximate standard error of the estimate is:

$$se_{\hat{\beta}_{1E}} = \sqrt{\frac{\sigma'^2}{\sum_{k=1}^{K} (X_k - \bar{X})^2 \sigma_{Yk}^{-2}}} \tag{19}$$

6.3.2 Statistical analysis

For the Frequentist analyses, the *MendelianRandomization* and *TwoSampleMR* packages were used. Some of the key functions used in both packages are listed in Tables 15 and 16 in the appendix. The *TwoSampleMR* package is the easier package to implement but is poorly documented. For this reason, the *MendelianRandomization* package was also used. This was done to ensure reproducible results were being obtained (by comparing the results obtained to ensure that they are the same).

In addition to the primary analysis, in which the causal effect of the exposure on the outcome is estimated, additional analyses were performed to assess model validity. These analyses can help assess the sensitivity of findings to instrument invalidity as well as invalidity that arises from horizontal pleiotropy. To demonstrate sensitivity to different patterns of assumption violations, a leave-one-out analysis, heterogeneity tests, and a funnel plot were used. The results of all of these can be found in the results section, below.

Heterogeneity tests

Heterogeneity in the causal effects between individual instruments is an indicator of potential violations of the IV assumptions. To assess the heterogeneity in our individual estimates, a Cochrane's Q test was performed on the results from the IVW and MR-Egger methods. Cochrane's Q test assesses whether multiple genetic variants provide similar causal estimates. If most variants provide similar results, the reliability of the conclusions are strengthened. However, the test is sensitive to slight differences in estimates as well as influential points and may therefore not be an accurate representation of the true heterogeneity among the estimates (Burgess et al., 2020).

Leave-one-out analysis

A leave-one-out analysis is used to evaluate whether the overall estimates obtained were biased by a single SNP that might have a particularly large influence in the model. The analysis is performed by re-estimating the overall effect by sequentially removing one SNP at a time. Identifying SNPs that, when removed, lead to a dramatic change in the estimate can also be informative about the sensitivity of the estimate to outliers (Burgess et al., 2020). The results from this analysis have been displayed in Figure 24.

Funnel plots

A funnel plot is a useful tool for assessing horizontal pleiotropy in which the estimate for a particular SNP is plotted against its precision. If there is asymmetry in the funnel plot, it may be indicative of a violation of the IV assumption that the variants must be independent of confounders of the exposure-outcome association, through horizontal pleiotropy (Burgess et al., 2020). The funnel plot is shown in 21.

6.3.3 Results

The results from both packages have been reported. The results from the *MendelianRandomization* package are shown in Table 10 and the results from the *TwoSampleMR* package are shown in Table 11. Both have been shown to demonstrate that the two packages produce the same results. The IVW method produced a smaller OR than the MR-Egger method. However, the IVW method produced a highly significant result, where the MR-Egger method produced a considerably less significant result. This OR is interpreted as an odds ratio per unit increase in BMI.

Table 10: Results from the Mendelian randomisation analysis using the *MendelianRandomization* package.

Method	**Estimate (log-odds scale)**	**Odds ratio**	**Standard error**	**p-value**	**Confidence Interval (Odds ratio)**
IVW	0.176	1.19	0.044	< 0.001	1.09, 1.30
MR-Egger	0.193	1.21	0.132	0.143	0.94, 1.57

Table 11: Results from the Mendelian randomisation analysis using the *TwoSampleMR* package.

Method	**Estimate (log-odds scale)**	**Odds ratio**	**Standard error**	**p-value**	**Confidence Interval (Odds ratio)**
IVW	0.176	1.19	0.044	< 0.001	1.09, 1.30
MR-Egger	0.193	1.21	0.132	0.143	0.94, 1.57

Table 12 Shows the results from a Cochrane's Q test for heterogeneity for each of the two models fitted. The results indicate heterogeneity in the individual effect estimates ($p < 0.001$) for both models. However, as seen in the funnel plot in Figure 21, there does not seem to be a strong pattern of asymmetry in the distribution of the individual effects. This means that the Mendelian randomisation results are unlikely to be invalidated by this test. The Cochrane's Q test also only tests that all estimates are the same, which means that only one estimate has to differ from one another for the null hypothesis to be rejected. Therefore, it does not necessarily indicate that there is heterogeneity in a large number of the estimates.

Table 12: Results from a test for heterogeneity.

Method	**Q**	**Q p-value**
Inverse Variance Weighting (IVW)	141.1	< 0.001
MR-Egger	141.2	< 0.001

Table 13 shows the results from a test for horizontal pleiotropy using the MR-Egger intercept. This involves testing whether the intercept term in the MR-Egger model differs from 0. The intercept in the MR-Egger model is interpreted as the average pleiotropic effect of a genetic variant. So testing whether the intercept term is significantly different from 0 is testing whether there is an indication of pleiotropy in the instrumental variables or not. The p-value shows no evidence that the intercept differs from 0 ($p = 0.89$), indicating a lack of evidence for horizontal pleiotropy which indicates that the instrumental variable assumptions relating to pleiotropy were probably not violated.

Table 13: Results from a test for pleiotropy.

Egger intercept	**Standard error**	**p-value**
-0.0004864	0.003561	0.8917

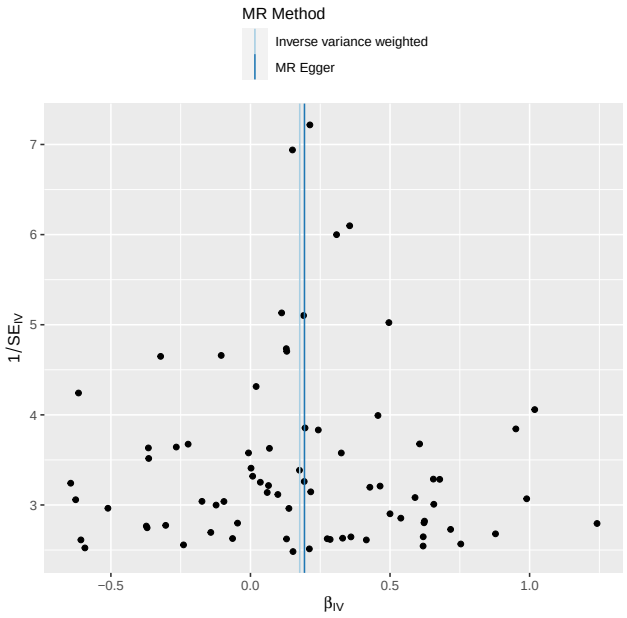
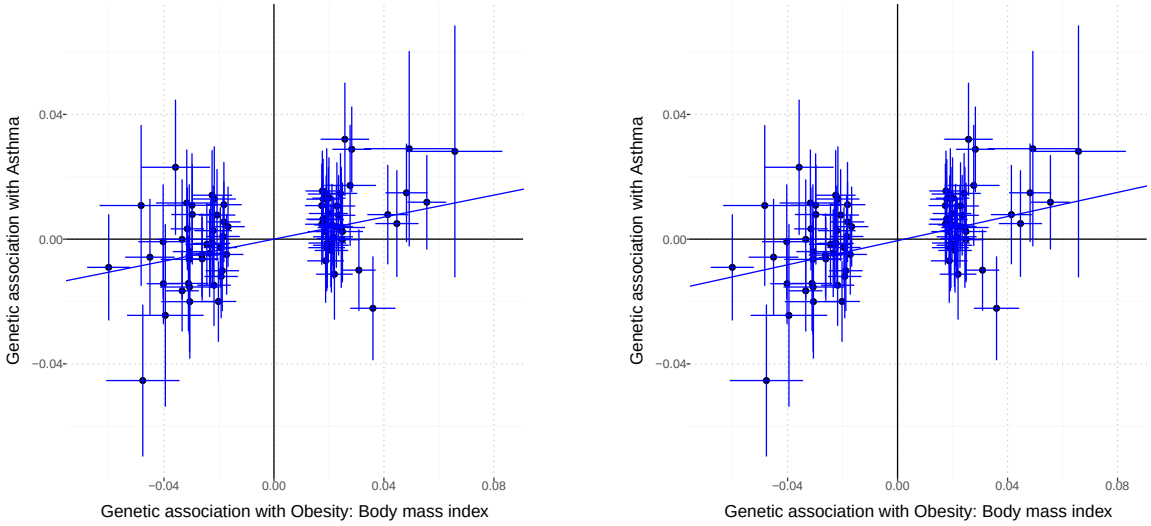


Figure 21: Funnel plot for assessing risk of pleiotropy in the included SNPs.

Figures 22a and 22b show the estimated genetic associations with the outcome (vertical axis) plotted against predicted associations with the exposure (horizontal axis). Each point on the graph represents a single genetic variant. The vertical and horizontal lines represent the 95% confidence intervals for the genetic associations with asthma and obesity respectively. The gradient of the line drawn through the origin on the graph represents the IVW estimate in Figure 22a and MR-Egger estimate in Figure 22b. These plots shows that some of the variants do not have the same directionality in their relationship with asthma as they do in their relationship with BMI. It also shows that most of the estimates which are strongly related to BMI are also relatively strongly related to asthma.



(a) Inverse-variance weighted (IVW) method

(b) MR-Egger method

Figure 22: Scatter plots of the genetic association with asthma (and 95% confidence interval)vs the genetic association with obesity (and 95% confidence interval) for each genetic variant.

Figure 23 shows the effect size for the effect of each individual SNP on asthma and also for the effect of BMI on asthma estimated from each of the two models (shown in red). The lines on either side of the point indicate the 95% confidence intervals for each estimate. Similar to the scatter plots in Figure 22, this plot indicates that the relationship between obesity and asthma is not the same over all the SNPs. Some of the SNPs indicate a positive relationship, some show close to no relationship and a few indicate a negative relationship. This does indicate some heterogeneity between the estimates for the different SNPs. This seems to agree with the Cochrane's Q test which also indicated heterogeneity in the estimates.

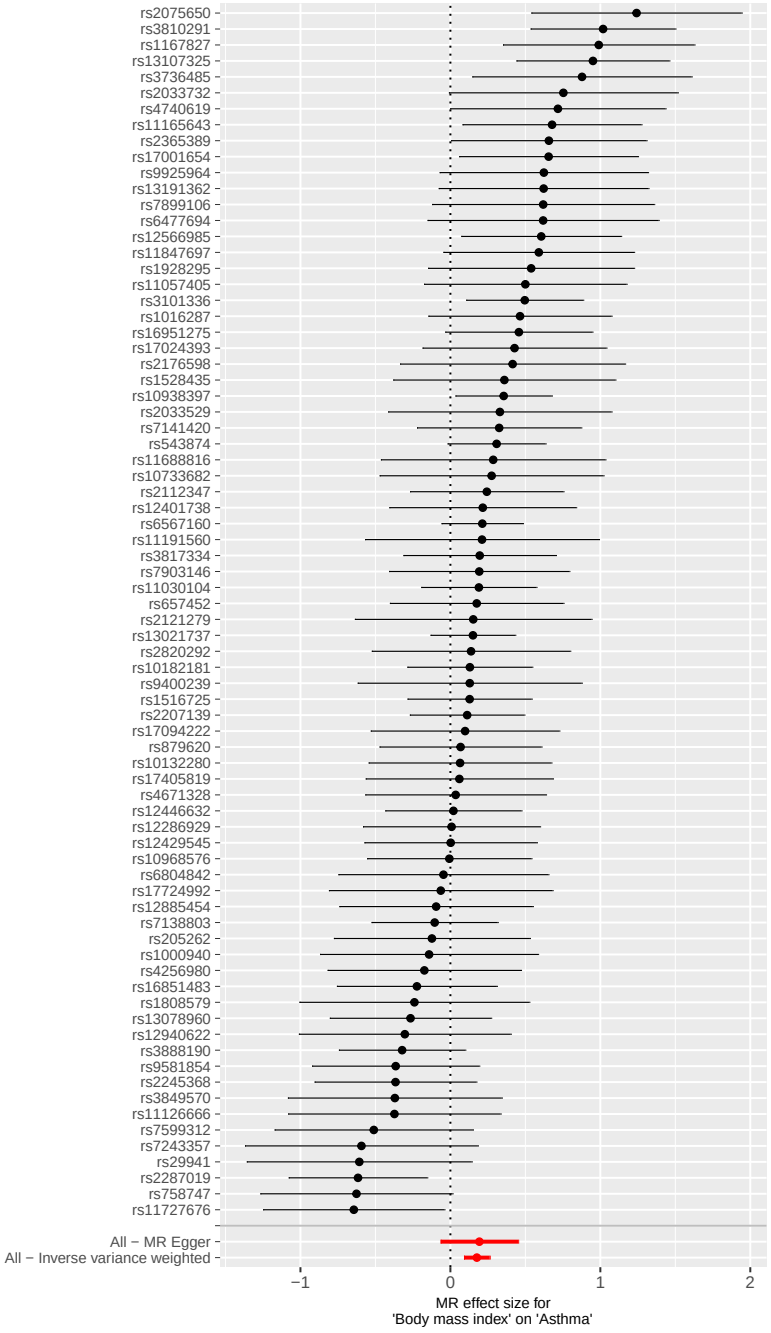


Figure 23: Forest plot of MR SNPs

A leave-one-out sensitivity analysis was conducted to investigate the influence of each SNP on the overall estimate. Figure 24 shows 77 IVW estimates, each one with a different SNP omitted. The SNP omitted in each estimate is shown on the left hand side of the plot. All of these estimates and their confidence intervals are extremely similar to each other and also to the IVW estimate based on all the SNPs (shown in red at the bottom of the plot). This indicates that none of the SNPs have high influence in the model. The two SNPs with the highest influence seem to be rs2287019 and rs3810291. rs2287019 has a relatively strong positive association with obesity but a relatively strong negative association with asthma. This would explain why the estimate increases when this SNP is removed. rs3810291 has a relatively strong association with obesity and asthma. This is why when removed from the analysis the estimate decreases slightly. These two most influential points are not of too much concern as they do not change the estimate by much.

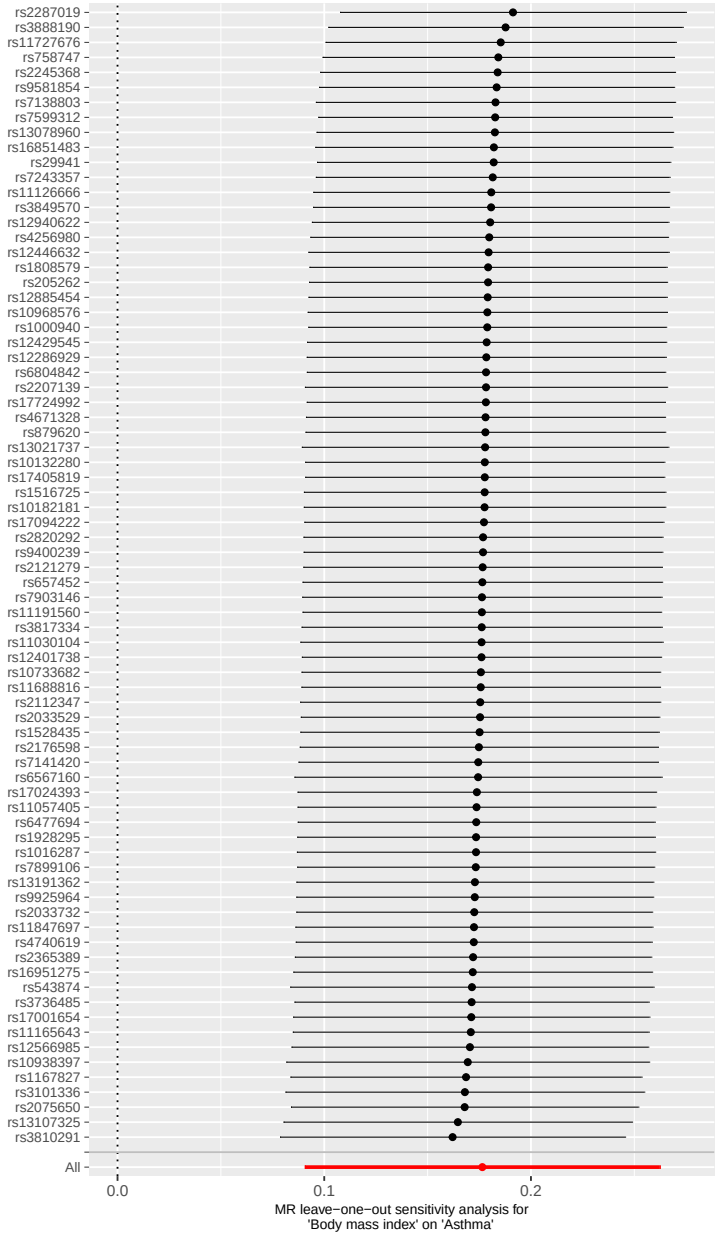


Figure 24: A plot of the leave-one-out analysis results for each variant, with the overall IVW estimate shown in red below for reference.

6.4 Bayesian Approach

These same methods from above were also implemented using a Bayesian approach. This was done as a comparison between the two approaches as well as to demonstrate the implementation for both of the main statistical paradigms.

6.4.1 Methods

The IVW method:

The model specification for the Bayesian IVW method is the same as for the Frequentist approach previously described. However, the parameters are alternatively treated as random instead of fixed. For the prior distribution of β_{IVW}, a standard normal distribution was used. This is because partial information was available from the literature, which allowed us to assume that the value is likely to be between -2 and 2 (on the log-odds scale). The distributions shown below are based on work by Uche-Ikonne, Dondelinger & Palmer (2020).

The chosen prior distribution is:

$$[\beta_{IVW}] \sim \mathcal{N}(0, 1) \tag{20}$$

The likelihood, assuming known variance $\sigma^2_{Y_k}$, follows a Normal distribution:

$$L(Y_k \mid \beta_{IVW}, \sigma_{Y_k}, X_k) = \prod_{k=1}^{K} \mathcal{N}(X_k \beta_{IVW}, \sigma^2_{Y_k}) \tag{21}$$

Therefore, the posterior distribution is:

$$P(\beta_{IVW} \mid Y_k, \sigma_{Y_k}, X_k) \propto [\beta_{IVW}]\, L(Y_k \mid \beta_{IVW}, \sigma_{Y_k}, X_k) \tag{22}$$

The Egger regression (MR-Egger) method:

As was the case with the model specification for the Bayesian IVW method, specification of the Bayesian MR-Egger model is the same as the Frequentist approach. However, parameters (β_{0E}, β_{1E} and σ') are now treated as random instead of fixed. For the prior distributions of β_{0E}, β_{1E} and σ', weakly informative distributions were used. This is because partial information is available based on expectation of the magnitude of these estimates and literature on the association. However, the data should still have a larger influence on the posterior distribution than the priors. All the distributions in this section are based on work by Uche-Ikonne, Dondelinger & Palmer (2020).

$$[\beta_{0E}] \sim \mathcal{N}(0, 1) \tag{23}$$
$$[\beta_{1E}] \sim \mathcal{N}(0, 1) \tag{24}$$
$$[\sigma'] \sim \mathcal{U}(0.0001, 10) \tag{25}$$

The chosen prior distributions for each parameter are shown above. A standard Normal distribution was chosen for both β_{0E} and β_{1E}, since the horizontal pleiotropy and overall estimate are both likely to lie between -2 and 2, but closer to 0, respectively. The residual standard deviation, σ', should be positive (and will never be exactly 0) and to be conservative the range extends to 10. This prior is mostly uninformative.

The likelihood, assuming known variance, follows a Normal distribution:

$$L(Y_k \mid \beta_{0E}, \beta_{1E}, \sigma', X_k) = \prod_{k=1}^{K} \mathcal{N}(\beta_{0E} + X_k\beta_{1E},\ \sigma'^2\sigma_{Y_k}^{-2}) \tag{26}$$

Therefore, the posterior distribution is:

$$P(\beta_{0E}, \beta_{1E}, \sigma' \mid Y_k, X_k) \propto [\beta_{0E}][\beta_{1E}][\sigma']\ L(Y_k \mid \beta_{0E}, \beta_{1E}, \sigma', X_k) \tag{27}$$

6.4.2 Statistical analysis

For our Bayesian analysis, we used the *mrbayes* package. Some of the key functions used are listed in Table 17 in the appendix. For both the IVW and MR-Egger model, 5000 iterations were used with a warm-up of 1000 and 3 chains. These models required fewer iterations than the three-level models used in the meta-analysis because they are simpler. This means that convergence is reached in fewer iterations. The traceplots in Figures 25 and 26 show that all the chains have converged (they are all stationary) and that the chains are mixing properly.

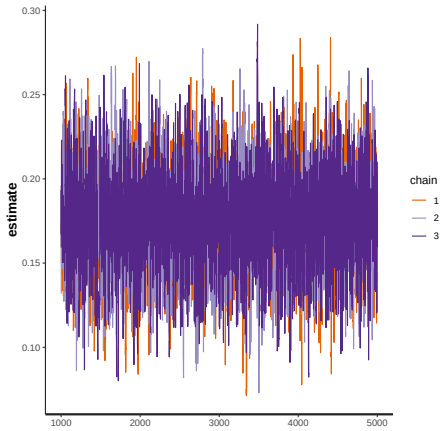


Figure 25: A traceplot of the IVW Estimate (Log-odds).

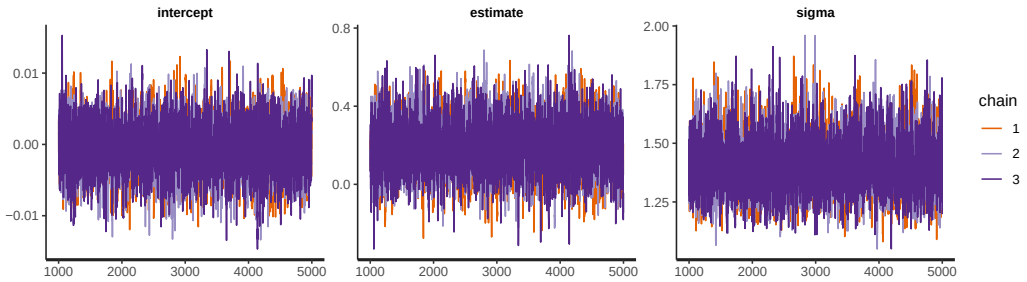


Figure 26: Traceplots of the MR-Egger estimates (Log-odds).

The only supplementary analysis conducted for the Bayesian approach was a leave-one-out sensitivity analysis. Similarly to the frequentist approach, this was done to assess the influence of each SNP in the model. The results from this analysis can be found in Table 29 in the results section, below.

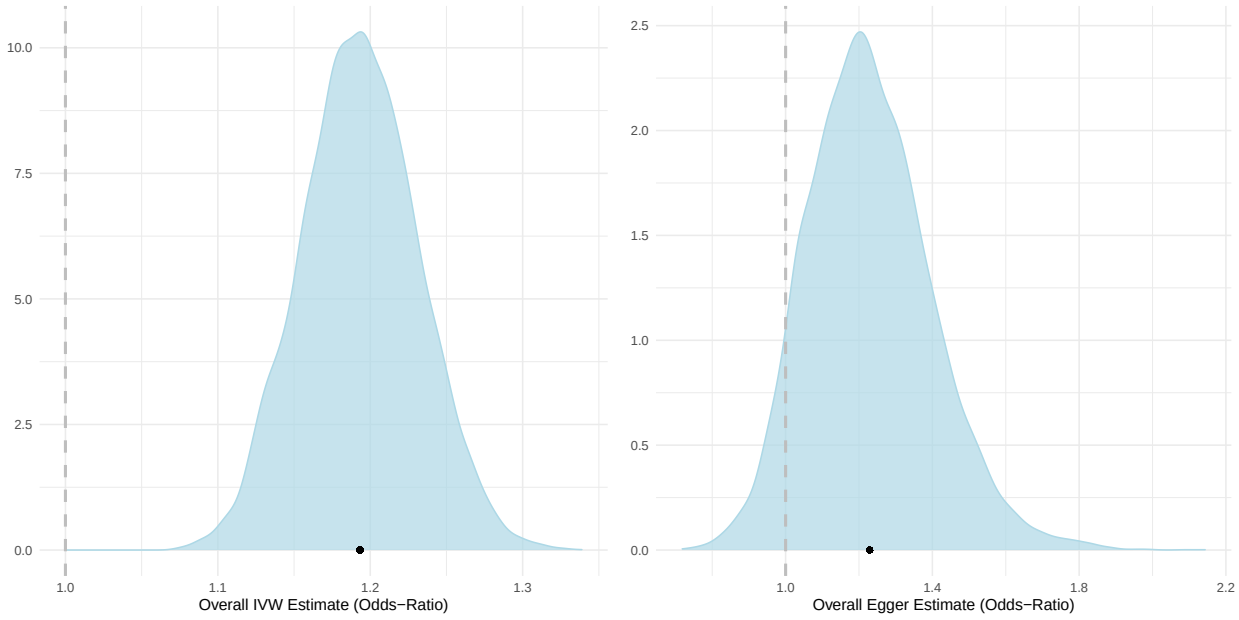
6.4.3 Results

In Table 14 below, the results from the Bayesian-based approach using the *mrbayes* package are displayed. The estimates are almost exactly the same as the estimates obtained using the frequentist approach (as seen in Table 10). The MR-Egger intercept estimate is 0 which combined with the very narrow credible interval indicates that there is no horizontal pleiotropy in the included SNPs. The IVW and MR-Egger estimates for the overall causal effect indicate a positive effect between obesity and asthma, although the IVW estimate is more confident than the MR-Egger estimate, since the credible interval does not pass through one (or zero on the log-odds scale).

Table 14: Results from the Mendelian randomisation using *mrbayes* package. All credible intervals were on a odds ratio scale except for the Egger intercept and sigma estimate.

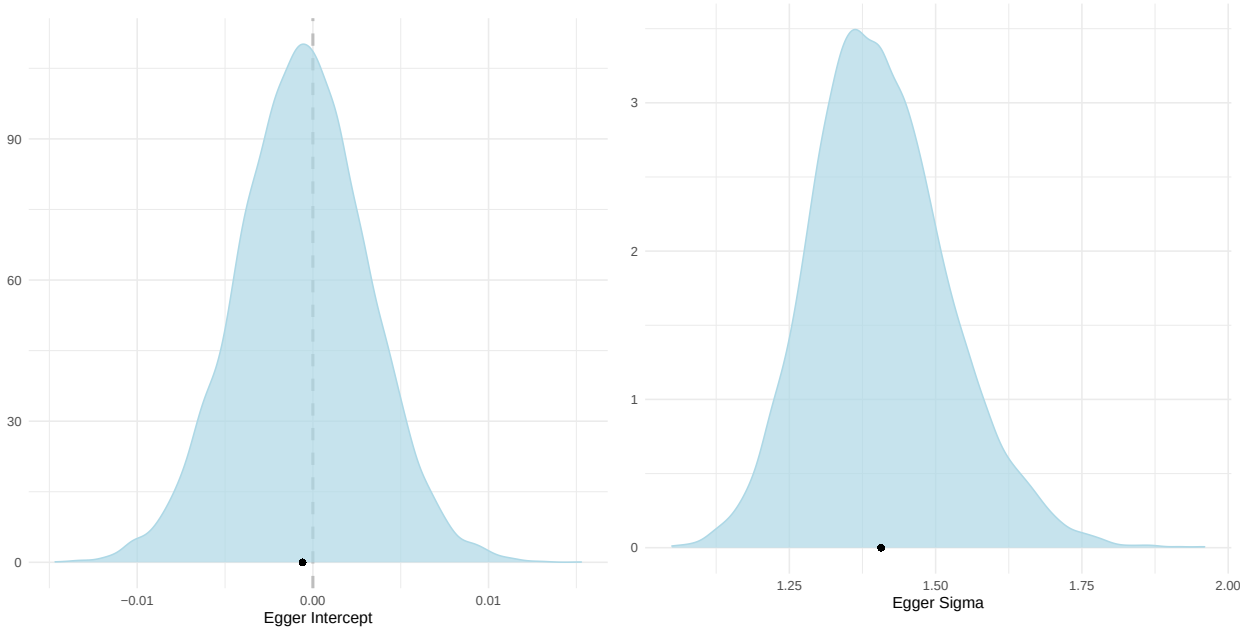
Method	**Estimate from model**	**Std Error**	**Odds ratio**	**95% Credible interval**
IVW Estimate	0.18	0.03	1.19	1.12, 1.27
MR-Egger Intercept	<0.01	<0.01		-0.01, 0.01
MR-Egger Estimate	0.20	0.14	1.22	0.94, 1.59
MR-Egger Sigma	1.41	0.12		1.20, 1.66

The posterior distributions for all of the parameters estimated are shown in Figures 27 and 28 below. These are all normally distributed and mostly symmetrical around the estimate. The distribution of the σ' estimate looks slightly skewed to the right meaning that higher values are slightly more likely than lower ones. For the estimates of the causal association in Figure 27, the dashed grey line shows the position of 1. The IVW distribution barely overlaps 1 (if it even does) where the Egger distribution does, quite clearly, overlap 1. This demonstrates, graphically, the difference in confidence associated with the two estimates. This is also reflected in the frequentist estimates and their confidence intervals.



(a) Posterior distribution of the overall IVW estimate. (b) Posterior distribution of the overall Egger estimate.

Figure 27: Plots of the posterior distributions of overall IVW and Egger estimates.



(a) Posterior distribution of the Egger intercept.

(b) Posterior distribution of the Egger residual standard error.

Figure 28: Plots of the posterior distributions of the Egger intercept and residual standard error.

The results from the leave-one-out sensitivity analysis are displayed in Figure 29. This does not indicate any influential SNPs as the estimates are all almost exactly the same. The most influential SNPs in this model are the same as in the frequentist approach (s2287019 and rs381029).

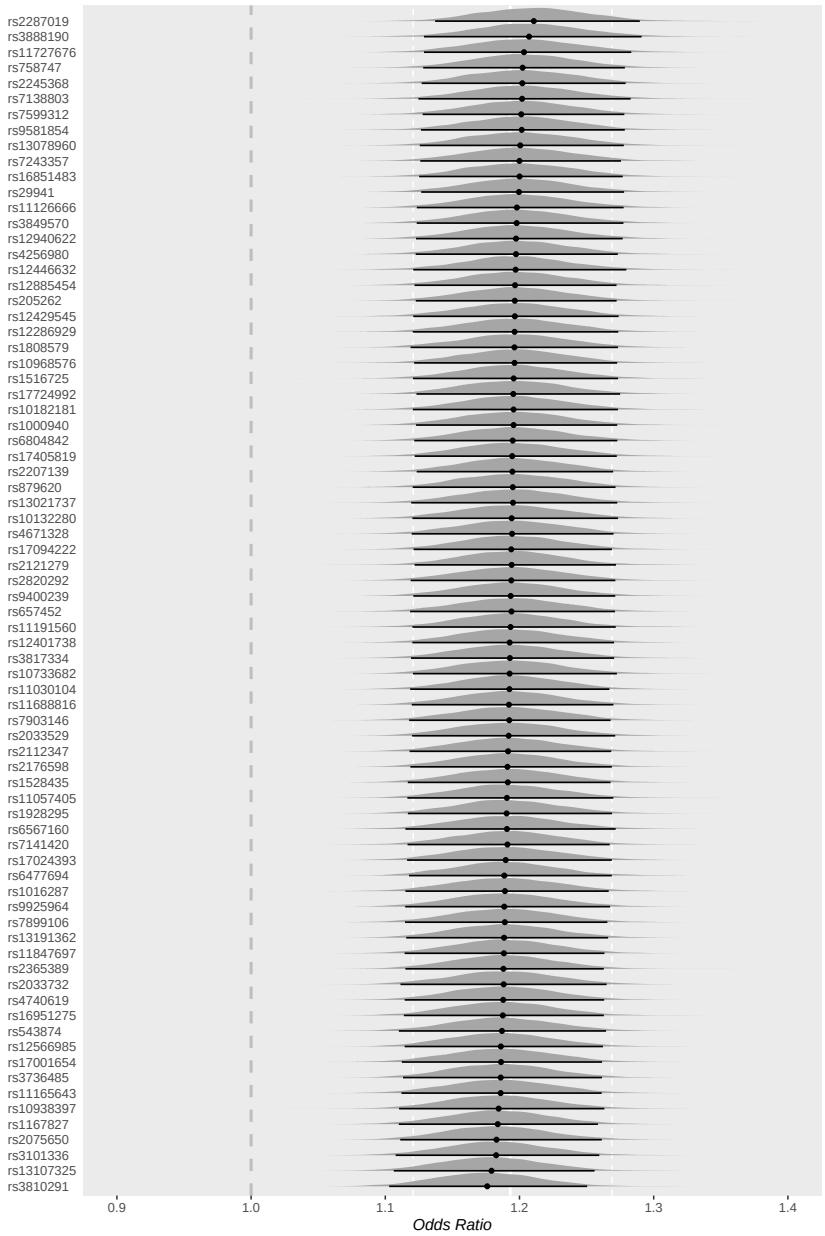


Figure 29: Results from the leave-one-out sensitivity analysis. The SNP listed on the left indicated the SNP left out in that model.

6.5 A Comparison of the Paradigms

The frequentist and Bayesian approaches produced almost identical results for the Mendelian randomisation. Both of these methods were relatively easy to implement, with the Bayesian models being slightly more complicated. The *mrbayes* package makes use of data in the input form of the *MendelianRandomization* package which makes these two packages quite complementary and nice to use for comparison. Both the frequentist and Bayesian approaches allow the direct modelling of pleiotropy in the MR-Egger models but only the Bayesian model allows the heterogeneity to be modelled. If there is substantial prior knowledge about the estimates, then a Bayesian approach is recommended. However, if not, then the choice of method is up to preference as one paradigm does not show clear advantages over the other.

7 Discussion

In this project, multiple methods have been used to demonstrate the use of publicly available summary data to asses causality, in addition to an association, between an exposure and outcome. In this case, the exposure is obesity, and the outcome is asthma. The first method involved summarising the available literature on the association between obesity and asthma in a systematic review to better understand the current information on the association. Six studies that met our criteria for inclusion were then selected from the pool of 18 studies used for the systematic review to be included in a meta-analysis. The meta-analysis then combined the information from these studies into a single overall estimate of the association between the exposure and outcome. The meta-analysis was then followed by a Mendelian randomisation, which also used summary data to obtain a single estimate of the association between the exposure and outcome using different models. The difference with this approach is that this estimate can be interpreted as a causal estimate, which is not the case in the meta-analysis. These three methods implemented in succession provide a holistic overview of the relationship between an exposure and outcome.

Two analysis approaches were used to implement the meta-analysis and Mendelian randomisation. An approach was implemented for the two main statistical paradigms, frequentist and Bayesian statistics. The Bayesian approach provides more insight into the heterogeneity in the models and allows the generation of posterior distributions. However, this approach is more computationally expensive than the frequentist approach. Both approaches have merit and can be used to produce a good-quality meta-analysis or Mendelian randomisation. Many statisticians prefer one paradigm over the other, therefore, there is a benefit in demonstrating the implementation of methods using both paradigms. It is also reassuring that both approaches produced very similar results. This demonstrates that for a meta-analysis and Mendelian randomisation with only little prior information about the parameters, either approach will produce an accurate estimate of the association under investigation. When there are stronger prior beliefs or the sample size included in the analysis is particularly small, the Bayesian approach may be a more accurate choice.

Within each of these analyses approaches, two different models were implemented for the Mendelian randomisation. The first was an inverse variance weighted (IVW) model and the second an MR-Egger model. The IVW model is the simpler of the two models and involves the calculation of an estimate of the slope of the relationship between the exposure and outcome. The MR-Egger model is a more robust implementation and also involves the estimation of an intercept and slope (with the intercept estimate the effect of pleiotropy in the model). The IVW estimate tends to be over-precise when there is imprecision in the individual estimates. This makes the MR-Egger model more appropriate when there is higher imprecision in the individual estimates, as it produces appropriately sized confidence intervals. When the estimates of the variant-exposure associations are all very similar in magnitude, the MR-Egger approach has lower precision. This can mean that the approach fails to detect a true causal association. The MR-Egger estimate is also greatly affected by outliers and influential data points. A limitation of both the IVW and MR-Egger approach is that they assume a linear relationship between the exposure and outcome. Therefore, if this relationship is more complex it will not be detected in either analysis. The only model used for the meta-analysis was a three-level mixed effect model. This model allows for the inclusion of multiple estimates from each study. A limitation of this approach in the frequentist application is that all estimates and studies as assumed to be sampled from an underlying normal distribution. If this assumption is violated, it would lead to unreliable results. However, the Bayesian application overcomes this as the underlying posterior distributions are generated for each within-study and each between-study estimate.

The relationship between obesity and asthma does not appear to be a clear and consistent one. This relationship is modified by sex and moderately by the age of asthma onset. The initial hypothesis was that there is a causal association with obesity causing asthma, as this is what previous research suggests. The results confirm this hypothesis; however, the MR-Egger model results indicate that this causal association may not be significant. This association would be more apparent when considering the modifiers mentioned above. The meta-analysis and Mendelian randomisation produced similar results, which indicates that the meta-analysis reflects the causal association between the traits. This similarity in results was unexpected. Due to the moderate bias in all of the studies included in the meta-analysis, the results were not expected to reflect the causal association identified in the Mendelian randomisation. Most of the findings in this paper

reflect the findings in previous research. Liu et al. (2013) conducted a systematic review on the association between obesity and asthma in children. Despite the study population being children, these results were similar to this systematic review. There was a positive association between obesity and asthma in the majority of the included studies. This study also indicated a difference in the association between sexes. Jiang, Wang, Bai and Chen (2019) conducted a meta-analysis to assess the association between obesity and asthma. Their results differ slightly to the results in this paper. The point estimates for the association are similar; however, their estimate was highly significant. Their methods involved fitting a fixed effect model and not a mixed effect model, which could possibly account for the differences. Xu, Gilliland and Conti (2019) conducted a Mendelian randomisation to explore the causal association between obesity and asthma. They only implemented an IVW approach but their estimate was almost exactly the same as the estimate here. The confidence interval was also extremely similar. It is important to not that this study made use of the same dataset for the variant-obesity associations as was used here. This probably contributed to the similarity of the results.

Data availability was a benefit and a limitation of this analysis. Summary data is easily accessible and available online, making it a convenient form of data. It also does not require complex data cleaning and handling, which can often be an issue when using non-summary data. However, summary data often results in too few studies available for inclusion in a meta-analysis. This is because it can be challenging to find a sufficient number of studies that report the same outcome measure for the same association. This is why only 6 of the 18 studies were included in the meta-analysis. This small number of studies is not uncommon. Most meta-analyses use similar, if not fewer studies. There is also the issue of access to research. Many online studies are not available for free. This restricts the pool of papers that are considered for inclusion if the study is not funded. The only studies accessible for this systematic review and meta-analysis were free, publicly available studies. This reduces the pool of information in the review and could bias the results. This also meant that the potential modifiers of the obesity-asthma association could not be explored. The systematic review indicated a possible difference in the association between obesity and asthma based on sex and age of asthma onset. Exploring these differences in the meta-analysis and Mendelian randomisation would have been interesting. However, this was not possible as there was insufficient availability of sex and age-stratified data for this analysis. The second major limitation was biological knowledge. Ideally, the instrumental variable assumptions should be assessed statistically and biologically for a Mendelian randomisation. In our case, only statistical tests were used.

Given the limitations above, an avenue for future research could be for someone with the relevant biological knowledge to attempt a Mendelian randomisation investigation of the association between obesity and asthma. This would allow for a better assessment of the instrumental variable assumptions required for the analysis. Another avenue for future research could be to attempt these methods using summary data stratified by age and sex, as mentioned in the limitations. This would allow the modification of the association by these factors to be assessed. The data necessary for this may be available in papers published in paid or subscription-based journals.

8 Conclusion

This paper demonstrates the use of a systematic review, meta-analysis and Mendelian randomisation to provide a comprehensive summary of the relationship between an exposure and outcome of interest. The two statistical paradigms (frequentist and Bayesian) were implemented and compared in the analyses to demonstrate the strengths and weaknesses of each approach. Ultimately the decision of which approach to implement is problem specific and can often be based on preference. All analyses indicated a possible relationship between obesity and asthma, and the Mendelian randomisation indicated that the direction of causality is that obesity causes asthma. However, these results were not highly significant in all the analyses and should be interpreted with caution. Hopefully, this paper has demonstrated the value of summary data in assessing an association between an exposure and outcome, and determining causality when randomised controlled trials are not possible.

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10 Appendix

All of the R code and data used to implement the methods in this paper can be found online at: `https://github.com/gracecarmichael/MA-and-MR-code`

Below are summaries of the characteristics of the included studies, as well as the risk of bias tables for each study.

10.1 Fitzpatrick et al. 2021

Methods	Longitudinal with baseline and 12-month follow-up. Participants classified as either lean, overweight, or obese according to age- and sex-specific body mass index (BMI) percentiles with the BMI-for-age percentile growth charts from the CDC
Participants	494 children 6 through 17 years of age with physician-diagnosed asthma at an academic specialty center for asthma in Atlanta, Georgia
Interventions	Overweight / obesity
Outcomes	asthma control, quality of life, lung function, and exacerbations
Outcome measures	Differences between BMI groups and associated p-values. Differences were measured using bio-markers and questionnaires.

Bias domain	**Signalling questions**	**Elaboration**	**Response**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Due to the study not being a randomized control trial, there is a high risk of confounding (measured or unmeasured). This study is no different and it would be prudent to assume that there is.	PY
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	No, follow up times were the same regardless of intervention received.	PN
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	The study mentions that differences between groups were compared for the different confounders, but additional information was not mentioned.	PN
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	There was no indication of controlling for post-intervention variables.	PN
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time- varying confounding?	There is no mention of any adjustments and the analysis method used does not account for this.	N
	Risk of bias judgement		Serious

Bias domain	Signalling questions	Elaboration	Response
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Selection of participants was based on characteristics observed before the start of intervention.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	The starting point and follow-up points seems to coincide for most participants, follow up was postponed if there was asthma exacerbation in the preceding two.	PY
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Groups were separated by their BMI levels as defined by the CDC (BMI by age charts).	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The information used to define intervention groups was recorded before the start of the intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	The classification status was determined by BMI as defined by the CDC.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	There were some complications due to asthma exacerbation before follow up, but these would also be expected in usual practice	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Nearly all of the participants had outcome data.	PY
	5.2 Were participants excluded due to missing data on intervention status?	There was no missing data with regards to intervention status.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	There's no indication that participants were excluded due to missing data.	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Knowledge of obesity / lean status would not influence the outcome measures	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	It is easy to visually see whether participants are obese or not and participants reported their outcomes themselves.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The outcome assessment was well defined and was the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Even though outcome assessors were aware of the interventions received, this is unlikely, since the outcome measurements were the same for all intervention groups. Misclassifications would therefore more likely be related to the method of outcome measurement and not the intervention received. *	PN
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All of the measurements of effect estimates that were made were reported.	PN
	7.2 ... multiple analyses of the intervention-outcome relationship?	The methods that were applied were reported on and all results from all the methods were reported.	PN
	7.3 ... different subgroups?	There were no subgroups *	NA
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.2 Barros et al. 2016

Methods	Cross-sectional. Data from the 4th Portuguese National Health Survey, carried out by the National Institute of Health and National Institute of Statistics (NIS), between 2005 and 2006, was analysed. WHO classification for obesity used.
Participants	The study included 32,644 adults, 52.6% female, from a representative sample of the 4th Portuguese National Health Survey.
Interventions	Obesity
Outcomes	Obesity defined by WHO and asthma (ever asthma (ever medical doctor asthma diagnosis), current asthma (asthma within the last 12 months), current persistent asthma (required asthma medication within the last 12 months), current severe asthma (attending an emergency department because of asthma within the last 12 months), and incident asthma (asthma diagnosis within the last 12 months)).
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Although the study mentions that confounders were adjusted for, there is still the possibility of unmeasured confounders, so the potential for confounding is high.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	This was a cross-sectional study and did not have a follow up time.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Logistic regression models that were adjusted for confounding were used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	There was self-reporting for variable such as age, weight and level of physical activity	PN
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	There was no indication of controlling for post-intervention variables.	PN
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?	This is not applicable to this study.	NI
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on who answered the survey.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Not applicable to this study, since there was no follow up.	NI
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	The obesity classifications were based on the WHO definitions	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Exposure was present before the start of the study.	N
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowledge of the outcome is highly unlikely to have effected the obesity classification.	PN
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Not applicable to this study.	NI
	Risk of bias judgement		No information

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	There were participants excluded but the study does not mention why they were excluded.	NI
	5.2 Were participants excluded due to missing data on intervention status?	This is not addressed in the study.	NI
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	There is no mention of excluding participants due to missing data on other variables.	NI
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The census data used was only looking at a single point in time, so the outcome could not be influenced by the knowledge of the intervention.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	All the data was available at the start of the study.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were identical for all intervention groups	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Errors in the asthma measurements were likely not due to obesity status	PN
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All outcome measurements were reported, without selection of a specific subset.	PN
	7.2 ... multiple analyses of the intervention-outcome relationship?	The methods used were pre-specified (adjusted logistic regression)	PN
	7.3 ... different subgroups?	All estimates relating to each subgroup were reported.	PN
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.3 Mosen et al. 2008

Methods	Cross-sectional. Outcomes included the mini-Asthma Quality of Life Questionnaire, the Asthma Therapy Assessment Questionnaire, and self-reported asthma-related hospitalization. Multiple logistic regression models were used to measure the association of BMI status with outcomes.
Participants	A random sample of 1113 members of a large integrated health care organization who were 35 years of age or older with health care use suggestive of active asthma.
Interventions	Obesity
Outcomes	Mini-Asthma Quality of Life Questionnaire, the Asthma Therapy Assessment Questionnaire, and self-reported asthma-related hospitalization
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Even though confounders were adjusted for, there is still a chance that some unmeasured confounders could have effected the results	PY
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	Participants could not switch between intervention groups	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multiple adjusted logistic regression models were used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	There was self-reporting, but the questionnaires used are designed in such a way to reduce error as much as possible.	PY
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	There was no indication of controlling for post-intervention variables, so it is unlikely that this occurred.	PN
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on characteristic observed before the start of intervention.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	The start of follow-up and that start of intervention was the same for most participants.	Y
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	The classification for being obese / non-obese was clearly defined.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The information regarding classification by BMI was recorded at the start of the intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowledge of the outcome would not have affected whether or not participants were classified as obese or not.	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	It is highly unlikely that there were deviations from what would be expected, since neither group would be able to change to the other due to the nature of obesity.	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	The outcome data indicates that there was outcome data available for nearly all of the study participants.	Y
	5.2 Were participants excluded due to missing data on intervention status?	There was no missing data on intervention status. Every participant was either obese, overweight or normal	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Unknown or missing values were excluded from the analysis, but not participants	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome measure, asthma status, was determined separately (by a doctor) from the intervention.	PN
	6.2 Were outcome assessors aware of the intervention received by study participants?	When the participants were selected based on their BMI and asthma status, the assessors were aware of who "received" the intervention or not.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The same methods of outcome assessment were used for all intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Any systemic errors in the outcome measurements would mainly be due to a misdiagnosis by the doctors and not due to the intervention.	PN
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	Every outcome measurement was reported.	PN
	7.2 ... multiple analyses of the intervention-outcome relationship?	Every result from every analysis was reported. Results were not left out.	N
	7.3 ... different subgroups?	Reported estimates for each subgroup were added. None were left out.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Low

10.4 To et al. 2018

Methods	Retrospective (case-control) observational study. The real-life data (clinical and laboratory data) of the enrolled patients were obtained from the electronic medical records. The characteristics of obesity and the metabolic functions are known to differ between males and females; therefore, we analyzed male-only and female-only cohorts separately.
Participants	A total of 492 Japanese patients were enrolled. Participants separated into two categories: obese and non-obese, defined by JASSO
Interventions	Obesity
Outcomes	Asthma (annual exacerbation ratio and the percentage of frequent exacerbators)
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	The study mentions that there may be confounding due to the effect of sex on BMI-defined obesity. There are also unmeasured confounders that could have a significant effect on the effect of the intervention.	PY
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	This was a retrospective observational study and did not have a follow up.	NA
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multivariate logistic regression was used to adjust for confounders.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	The study mentions that there were confounders, but specific details on how the variables were measured were not measured.	NI
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Post-intervention variables were not controlled for.	PN
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time- varying confounding?	Not applicable to this study.	NA
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on their asthma status and were obese / non-obese (the intervention) before they had an asthma status, so selection is based on asthma status, which occurred after the intervention	Y
	2.2. If Y/PY to 2.1: Were the post-intervention variables that influenced selection likely to be associated with intervention?	Asthma status is associated with obesity.	PY
	2.3 If Y/PY to 2.2: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	It was the outcome that was determined the selection of participants.	Y
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Participants were selected continuously for a month and follow up occurred afterwards, so they do not coincide.	N
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	There was no mention of adjustment techniques for selection biases	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were defined by JASSO (obese and non-obese)	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The intervention groups were defined before the start of the intervention	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Although there was outcome knowledge, the intervention status of participants was determined independently of the outcome, so it would not have affected it.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Participants could not switch treatment groups and there was no follow-up, so deviations would be similiar to those expected in usual practice.	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	The number of participants selected for the study and the number for which outcome measures were provided were the same.	PY
	5.2 Were participants excluded due to missing data on intervention status?	Intervention status was known for all of the participants	PN
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants were excluded if they did not adhere to asthma medication guidelines.	Y
	5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	The number of participants excluded were similiar across interventions. The proportions were also similiar.	Y
	5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	There was no indication that missing values were imputed or that a sensitivity analysis was performed	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Participants were selected based on their asthma status which was determined independently, so knowledge of the intervention status would not have affected the outcome measure	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	The outcome assessors had access to the data and were aware of the intervention received by all participants.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were that same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Since the outcome measurements were done independently of the intervention groups (participants were selected based on their outcome status), it is not possible that systematic errors were related to intervention received.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All of the outcome measurements were reported and discussed, without specifically selecting effect estimates.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	From the study, it seems that only multivariate logistic regression was used and all of the effect estimates from that analysis were reported.	PN
	7.3 ... different subgroups?	The two subgroups were male and female and every effect estimate generated was selected and reported on.	PN
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.5 Green 2012

Methods	Longitudinal cohort study
Participants	The Early Childhood Longitudinal Study-Kindergarten Class of 1998–1999(ECLS-K) is a nationally representative, longitudinal study that follows a cohort of children from kindergarten to middle school.
Interventions	Overweight and obesity
Outcomes	Asthma ever, new asthma, asthma treatment, new treatment
Outcome measures	APE (average partial effects)

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	This is an observational study, meaning that there is always the potential for confounding. The way participants were selected also indicates that there is potential for confounding.	PY
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	The follow-up time was the same and was not split according to intervention status	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	The study mentions that logit regression as well as OLS regression was used to account for confounders.	PY
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	The confounding variables seem to have been measured accurately and reliably and the information relating to them was explained.	PY
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Only pre-intervention variables were adjusted for.	PN
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?	There was no indication that time-varying confounding was adjusted for.	PN
	1.8. Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	The data for the study seems to have been measured reliably.	PY
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	The selection of participants was based on characteristics observed before the start of intervention.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	The start of intervention and follow-up seems to coincide based on the information provided.	PY
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were based on BMI-for-age status.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The BMI was recorded before the start of intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Intervention status was based on BMI alone.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	The data suggests that some participants changed intervention groups over the course of the study (became obese, overweight or normal weight)	Y
	4.2. If Y/PY to 4.1: Were these deviations from intended intervention unbalanced between groups and likely to have affected the outcome?	The proportion of participants switching between groups was unbalanced and likely affected the outcome	Y
	Risk of bias judgement		Serious

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Of the participants who were included, nearly all of them had outcome data available.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Intervention status was known for all participants.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants who did not have a valid birth weight, valid race and gender data etc. were excluded.	Y
	5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	The proportions of participants with missing data and reasons for missing data were similiar across interventions.	Y
	5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	The study mentions that weights were used in the regression to account for missing data.	PY
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome measure (asthma status) was determined independently of BMI status.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	The outcome assessors knew which participants received which intervention.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were the same across intervention groups	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Since the asthma outcomes were determined independently, it is unlikely that systemic errors in outcome measurement were related to the intervention received.	PN
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All of the effect estimates were reported for each outcome domain.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	Although there were multiple analyses, the effect estimates for each one was reported.	N
	7.3 ... different subgroups?	No subgroups were analysed	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Serious

10.6 Lang et al. 2011

Methods	Longitudinal study (mixed-effect model)
Participants	490 patients with mild persistent asthma taken from 2,794 study visits from a prospective trial studying strategies of step-down therapy
Interventions	Obesity
Outcomes	asthma characteristics, including: spirometry, asthma control, airway pH, and perception of airflow changes
Outcome measures	Least square mean with p-value

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Placing participants into groups based on their BMI (not randomly assigning, since this is not possible) increases the potential for confounding.	PY
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	The follow up time for all intervention groups was the same.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	A mixed-effect model that controlled for confounders was used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Data on confounders was collected using questionnaires and backed up by lab-results.	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	There was no mention of controlling for post-intervention variables.	N
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?	A longitudinal mixed-effect model was used to account for the confounding domains.	Y
	1.8. Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	Mentioned above	Y
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on their asthma status and were already obese / lean at the time of selection, so this was after the start of the intervention.	Y
	2.2. If Y/PY to 2.1: Were the post-intervention variables that influenced selection likely to be associated with intervention?	Asthma is likely to be associated with the intervention.	PY
	2.3 If Y/PY to 2.2: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	It was the outcome.	Y
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Follow-up coincides for most participants.	Y
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?		N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were clearly defined by their BMI status.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Intervention groups were defined at the start of the study.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowledge of the outcome would not have affected which intervention group (based on BMI) participants were placed into.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	It does not seem that participants switched intervention groups during the study or that there were any other deviations that would not be expected.	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Although not individual data, but the number of participants for which there is outcome data is nearly the same as the number at the start of the intervention.	PY
	5.2 Were participants excluded due to missing data on intervention status?	Every participant had data relating to their BMI available.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Missing data was imputed.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Knowledge of being obese would not have influenced the asthma outcome (clinically diagnosed)	PN
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors knew which participants were obese / non-obese	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were exactly the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	The outcome measures were based on a clinical diagnosis done independently. Participants were chosen based on this measure.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	There were multiple asthma outcome measures, all of which were reported and discussed.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	There were multiple analyses of the intervention-outcome relationship, but none seemed to be chosen and selectively reported on above the others.	N
	7.3 ... different subgroups?	There were multiple subgroups (defined by age ranges), but the results from each were reported and elaborated on.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.7 Holguin et al. 2011

Methods	Cohort study (retrospective)
Participants	The study population consisted of participants aged 18 years or older from the multicenter SARP study that met the criteria for asthma, which included either a 12% increase in FEV after a short-acting bronchodilator or a 20% decrease in FEV after inhalation of methacholine
Interventions	Overweight and obesity
Outcomes	Asthma severity and duration
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Participants were selected based on their asthma status and were then divided into their respective intervention groups defined by BMI. This process was not random, so it is highly likely that the effect of the intervention would be confounded.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	The follow up time was the same for each intervention group.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multivariate logistic regression was used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Data was obtained using questionnaires and from the Severe Asthma Research Program.	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Only pre-intervention variables were adjusted for.	N
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?	Multivariate logistic regression was used.	Y
	1.8. Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	Allergy tests, bio-marker tests and questionnaires were given to participants	Y
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	The participants were selected based on their asthma status, which was observed after the intervention (obesity)	PY
	2.2. If Y/PY to 2.1: Were the post-intervention variables that influenced selection likely to be associated with intervention?	Obesity is associated with outcome	Y
	2.3 If Y/PY to 2.2: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	Participants were selected based on the outcome measure.	Y
	2.4. Do start of follow-up and start of intervention coincide for most participants?	The start of intervention and follow up were the same for participants.	Y
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	There was no mention of adjustment techniques used to correct for selection biases.	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	The intervention groups were clearly defined by their BMI levels	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The BMI groups were defined at the start of intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	The BMI status would not have been affected by knowledge of outcome, since the BMI measurements were done independently	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Some individuals became obese and some became lean over the course of the study, which means that intervention groups were changed. This would be classified as an unexpected deviation.	PY
	4.2. If Y/PY to 4.1: Were these deviations from intended intervention unbalanced between groups and likely to have affected the outcome?	There were very few deviations and they were balanced between the groups. It is unlikely to have affected the outcome.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Outcome data were available for all of the participants.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Intervention status was known for all of the participants.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No participants were excluded due to missing data.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome measure was assessed independently and would not have been affected by knowledge of the intervention received.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors knew who was in which intervention group	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were the same across treatment groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Systemic errors would be a result of an incorrect asthma diagnosis and not related to the intervention received.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	Every outcome measurement was reported and discussed. No measurements seemed to have been selected specifically.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	There were multiple analyses, but each one of them was reported and discussed.	N
	7.3 ... different subgroups?	All of the reported effect estimates for the different age subgroups were displayed and discussed.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Serious

10.8 Forte et al. 2013

Methods	Cross-sectional
Participants	272 patients aged 11 years and older with confirmed asthma from the outpatient asthma clinic of Hospital de Clínicas de Porto Alegre, Brazil
Interventions	Overweight and Obesity
Outcomes	Asthma control and severity
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Participants were selected based on their asthma status and were then grouped by their BMI statuses, which is not a randomised process.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	This was a cross-sectional study, so follow-up is not applicable	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Logistic regression was used to control for confounders.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Many of the measures were lab results and those that weren't were verified, so the measurements were valid and reliable.	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	There was no mention of controlling for post-intervention variables.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on their asthma diagnosis, which is the outcome, but since this is a cross-sectional study, the intervention and outcome occurred at the same time.	PN
	2.4. Do start of follow-up and start of intervention coincide for most participants?	There was no follow-up	NA
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were defined by BMI status.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The BMI levels used to classify participants was recorded at the start of the intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	The BMI level of participants would not have been affected by knowledge of asthma status.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Participants could not switch between intervention groups in this study, so any deviations would be expected.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Outcome data were available for nearly all study participants.	Y
	5.2 Were participants excluded due to missing data on intervention status?	The BMI status for all participants was known.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants were excluded if they did not complete all of the evaluations required by the protocol.	Y
	5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	The proportions and reasons for missing data were the same across intervention groups.	Y
	5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	There was no indication that the results were robust to missing data.	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The asthma diagnosis was done by a physician and would not have been influenced by knowledge of intervention received.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors were aware of intervention received by participants.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Systematic errors in the measurement of the outcome would be related to an error by a physician and not the intervention received.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All outcome measurements were reported and discussed	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	All of the effect estimates for each of the analyses were provided and discussed.	N
	7.3 ... different subgroups?	There were no subgroups	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Serious

10.9 Luthe et al. 2018

Methods	Retrospective cohort study
Participants	Population-based data of 8 geographically diverse US states from 2010 through 2013. The data included adults (age 18-54 years) hospitalized for asthma exacerbation.
Interventions	Obesity
Outcomes	markers of acute severity: use of mechanical ventilation (defined by noninvasive positive pressure ventilation and/or invasive mechanical ventilation) and length of hospital stay
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	The patients were selected based on their asthma status and were then divided into their respective intervention groups based on their BMI. Since there was no random assignment to intervention group, it is highly likely that confounders were not accounted for.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	The follow up time for all intervention groups were the same.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multivariate adjusted logistic models were used to deal with confounding.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	The data came from a database managed by the Agency for Healthcare Research and Quality	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Only pre-intervention variables were controlled for.	N
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?	Inverse probability weighting was used to account for time varying confounding and adjusted logistic regression models were used for the other confounding domains.	Y
	1.8. Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	The data for the variables came from a high-quality medical database and the confounding domains were measured validly.	Y
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on whether they had been hospitalised for asthma exacerbation, so the researchers were trying to determine the association between obesity and asthma that leads to being hospitalised.	PN
	2.4. Do start of follow-up and start of intervention coincide for most participants?	The start of intervention and follow up were the same for most of the participants.	Y
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were defined by ICD-9-CM, which is highly reliable.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The information used to define intervention groups was recorded before the start of intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Classification of intervention status was done independently and would not have been affected by the knowledge of the outcome. This is because obesity was well-defined.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Participants did not switch intervention groups during the study (obesity status did not change) and other unexpected deviations did not occur.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Outcome data was available for most participants.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Patients who were underweight were excluded.	Y
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants were excluded if they were 55 or older or left the hospital early or sex wasn't recorded	Y
	5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	The people who were underweight would not be included, so it is not equal across treatment groups.	N
	5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	A sensitivity analysis was conducted to ensure that the results were robust.	Y
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome was determined independently by a physician and would not have been affected by intervention received.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	In this study, the outcome assessors had access to the entire dataset.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were exactly the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Systematic error would be related to a misdiagnosis by the physician and not the intervention received.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All results were reported and discussed. There was no clear indication that certain results were selected over others.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	Every analysis was explained and the results reported.	N
	7.3 ... different subgroups?	Subgroups were not analysed.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Serious

10.10 Gonzalez-Barcala et al. 2012

Methods	Cross-sectional. Questionnaires were filled in by parents with questions about asthma status and severity.
Participants	Two groups of children, one with 6-7 year-olds (young children), and another with 13-14 year-olds (adolescents), from six of the main Health Areas in Galicia (Spain)
Interventions	Overweight/obesity
Outcomes	wheezing ever, current asthma, exercise-induced asthma and severe asthma
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	This is not a randomized controlled trial so there are many possible confounders.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	Cross-sectional study.	NA
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multivariate logistic regression was used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	The data used was validated externally, though it was based on self-reported answers to questionnaires.	PY
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Authors did not control for any post-intervention variables, only pre-intervention variables.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were chosen based on who filled out the questionnaires.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	They were the same for most participants	Y
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	BMI for age and sex was used to define intervention groups.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Obesity status was present before the start of the study so was only recorded after the start of intervention.	N
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowledge of the asthma outcome would not have affected the classification of the intervention, since the BMI cut-off were determined independently	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	There was no switching between intervention groups, so any deviations would be similiar to those expected from a randomized trial.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	The authors mention that incomplete surveys were excluded but not how they were incomplete.	NI
	5.2 Were participants excluded due to missing data on intervention status?	Participants were excluded due to missing data, which could have been missing data needed to calculate the BMI.	PY
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants were excluded due to any missing data from incomplete questionnaires.	PY
	5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	No information on the exact proportions or number of participants excluded due to missing data was provided.	NI
	5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	There was no indication that a sensitivity or similiar analysis was conducted to ensure that the results were robust to missing data	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome measure was determined independently and	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors had access to the entire data set and therefore knew of the intervention received by participants.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were exactly the same across all treatment groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Any systematic errors in outcome measurement would be related to incorrect information provided by participants and not the intervention.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All outcome measurements were reported.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	There was only one analysis of the intervention-outcome relationship.	N
	7.3 ... different subgroups?	There were two subgroups of ages that were analysed and the results from both were reported and discussed.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.11 Wang et al. 2015

Methods	Cross-sectional study
Participants	2012 National Health Interview Survey (n 33,153 adults aged 18 years or older, 4197 had asthma)
Interventions	Obesity: class I (BMI 30-34.9), class II (BMI 35-39.9) and class III(BMI $\geq$40)
Outcomes	Asthma
Outcome measures	Prevalence and Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Although participants were randomly selected from survey data, assignment to the intervention was not random from that sample, which means that there is likely confounding.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	Cross-sectional study.	NA
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multivariate logistic regression was used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	The data came from a national health survey, so it should be valid and somewhat reliable.	PY
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No post-intervention variables were controlled for.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were randomly selected based on their age and were then divided into their respective exposure groups.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Cross-sectional study	NA
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	There was no indication that techniques such as inverse probability weighting etc were used.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were clearly defined according to BMI status.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The BMI information was defined at the start of the intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Classification of intervention status was done according to BMI alone and would not have been affected by knowledge of outcome.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	No participants switched between intervention groups and there was no indication that there were any other unexpected deviations.	PN
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Outcome or exposure data was missing for over 1000 participants.	N
	5.2 Were participants excluded due to missing data on intervention status?	Outcome or exposure data was missing for over 1000 participants.	Y
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	There is no mention of exclusion due to other variables.	NI
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome measure was asthma status, which was determined by a physician and would not have been affected by knowledge of the intervention.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors had access to all of the data for the analysis and were aware of interventions received by participants.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	They were the same for all intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Any systematic error in the measurement of the outcome would be related to incorrect information supplied by the participants or a misdiagnosis by a physician.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	There was only a single outcome measurement.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	The results from every analysis were reported and discussed.	N
	7.3 ... different subgroups?	The results for each subgroup were reported and discussed.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.12 Jeong et al. 2017

Methods	Longitudinal cohort study (retrospective)
Participants	Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA) recruited 9651 adults aged 18-62 years. 8047 subjects participated in the first follow-up and 6088 in the second follow-up
Interventions	BMI, percentage body fat, waist-hip ratio, waist circumference and waist-height ratio
Outcomes	4 Asthma classes: persistent multiple symptom-presenting asthma, symptom-presenting asthma, symptom-free atopic asthma and symptom-free non-atopic asthma
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	The intervention is obesity and there are many possible confounders of the effect of obesity.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	The follow-up times were at set dates and not based on intervention group.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	The authors controlled for 5 confounders in the regression model. There may possibly be more.	PY
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	All confounding variables were easily measured and were therefore measured validy. The measurements seemed reliable as the same measurement tools were used across all participants.	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	All of the variables controlled for were assessed pre-intervention	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Selection of participants was determined by which participants answered the questionnaire and was therefore not based on any characteristics. All participants who attended a second visit were included in the analysis.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Due to the nature of the intervention (obesity) it is present before the follow-up.	N
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	There is no mention of selection bias in the paper.	PN
	Risk of bias judgement		Serious

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	5 obesity measures were used to classify individuals as obese or non-obese.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The initial asthma questions and obesity measures were assessed at the same time	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowing asthma status would not affect obesity status.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	The only possible deviation from intervention could be weight loss or weight gain which is unlikely in the study duration and is expected in usual practice	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Some participants did not attend a second follow-up and were therefore not included but there was still a decent number of participants.	PY
	5.2 Were participants excluded due to missing data on intervention status?	There was intervention status for all participants.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants were only excluded due to lack of outcome data from the second follow-up.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Asthma cannot be influenced by knowledge of obesity.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	The data was collected as part of a questionnaire so the intervention and outcome were not defined when the data was collected.	N
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The outcome assessment is the same for all participants.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	There were no systematic errors in measurement of outcome.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	The outcome measurements are just 4 classes of asthma and estimates are reported for all four.	N
	7.2 ... multiple analyses of the intervention- outcome relationship?	Only adjusted regression model estimates are generated and reported.	N
	7.3 ... different subgroups?	There are no subgroups used in the analysis.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.13 Xu et al. 2016

Methods	Longitudinal cohort study (with 3 visits)
Participants	Recruited from urban primary care clinics a prospective cohort of nonsmoking individuals with asthma who were 60 years or older without a history of other respiratory diseases.
Interventions	Overweight (BMI 25-30) and obesity (BMI $\geq$ 30)
Outcomes	Asthma control (measured using Asthma Control Questionnaire, ACQ), asthma quality of life (Mini Asthma Quality of Life Questionnaire, Mini-AQLQ) and asthma-related resource utilization (self-reported inpatient resource utilization)
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Since intervention groups were not randomly assigned, it is highly likely that there is unmeasured confounding.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	Follow up times were the same for all intervention groups.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Generalized estimating equations were used that adjusted for confounders.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	All participants underwent standardized in-person interviews to collect the necessary data.	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No post-intervention variables were controlled for.	N
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time-varying confounding?	There was no mention of adjusting for time-varying confounding	PN
	1.8. Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	The participants completed questionnaires that were later validated.	Y
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on their asthma status and were then divided into their treatment groups, which were defined at the start of intervention.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	The start of intervention and follow-up were nearly the same for all participants.	Y
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were clearly defined (BMI groups)	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The intervention groups were defined at baseline	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowledge of outcome would not have affected BMI classification.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	No participants switched intervention groups, so any deviations would be similar to those in an RCT.	PN
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Outcome data were available for all participants, since they were selected based on outcome.	Y
	5.2 Were participants excluded due to missing data on intervention status?	The intervention status was known for all participants.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants were not excluded due to any variables that are required for the analysis.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome measurement would not have been affected	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors were aware of which participants were assigned to which intervention groups.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	Methods of outcome assessment were the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Any systematic errors would be related to a misdiagnosis of asthma by a healthcare professional and not the intervention received.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All outcome measurements were reported and discussed.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	All results from every analysis were provided and explained.	N
	7.3 ... different subgroups?	There were no subgroups	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Low

10.14 Okubo et al. 2016

Methods	Retrospective cohort study
Participants	Hospital discharge records of patients aged 2 to 18 years with a diagnosis of asthma were obtained from the 2012 Kids' Inpatient Database, wherein the data were compiled by the Agency for Healthcare Research and Quality. To classify whether the patient was obese or not, the International Classification of Diseases, Ninth Revision, Clinical Modification code 278.0x was used.
Interventions	Obesity
Outcomes	Total charge for inpatient service in2012 US dollars, length of hospital stay, and use of mechanical ventilation (noninvasive or invasive)
Outcome measures	Odds ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	Patients were selected based on their asthma diagnosis and were then categorized into intervention groups. Since groups were not randomly assigned, this means that unmeasured confounding is likely to have an effect on the intervention.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	The follow-up time for all intervention groups was the same.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multivariate logistic regression was used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	The data for the confounding domains came from a large, nationally representative database, KID.	PY
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No post-intervention variables were controlled for.	N
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on their asthma diagnosis and assigned to an intervention group.	PN
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Start of follow-up and intervention were the same for all participants in the study.	Y
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were clearly defined using ICD-9CM codes 278.0x	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The information used to define intervention groups was available before the start of intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowledge of the outcome would not have affected the classification of intervention status.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Participants were not able to switch intervention groups, so any deviations would be expected.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Outcome data were available for all participants.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Intervention status was known for all participants.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Participants were only excluded if they chronic health conditions other than asthma.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Knowledge of the intervention received would not have affected the outcome measure, since the outcome measure is a clinical diagnosis.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors were aware of the intervention status of all participants.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were the same for all intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Systemic errors would likely be caused by a misdiagnosis and not the intervention received.	PN
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	Every outcome measure was reported and discussed.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	The results from every analysis were reported and discussed.	N
	7.3 ... different subgroups?	There were no subgroups.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.15 Ho et al. 2011

Methods	Longitudinal study with 2 time points
Participants	4,052 adolescents (aged 13-15) with undiagnosed asthma-like symptoms at baseline
Interventions	Weight category (underweight, normal, overweight and obese)
Outcomes	Developing asthma from pre-asthmatic symptoms
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	There are many possible confounders of the effect of obesity	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	Follow up times were mostly the same and were split over days randomly, not according to intervention.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Logistic regression was used and the model was adjusted for 10 possible confounders.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	All the confounding domains werre easily measured.	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No post-intervention variables were controlled for.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Selection into the study was random from the recorded survey.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Due to the nature of obesity it is present before the start of follow-up.	N
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	There is no mention of adjustments made.	N
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Groups were split based on BMI which was well defined for age group and sex.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The intervention started before the start of the study or survey.	N
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Obesity status does not changed based on knowledge of asthma.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	There were no deviations from intended intervention as BMI was computed at the start of study and follow-up.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	No participants were excluded due to missing outcome data.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Some participants were excluded due to missing data in important analytical variables which colud be intervention status.	PY
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	As stated above, participants were excluded due to missing data needed for analysis.	Y
	5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	Authors report that proportions were not significantly different.	Y
	5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	There is no mention of adjustments due to missing data.	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Knowledge of obesity status does not affect asthma status.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	The data was recorded as part of a national survey and therefore, assessors did not know what the intervention was when outcome was assessed.	N
	6.3 Were the methods of outcome assessment comparable across intervention groups?	They were exactly the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	There were no systematic errors in the measurement on the outcome.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	There was only a single outcme measurement.	N
	7.2 ... multiple analyses of the intervention- outcome relationship?	There were two models fitted but both were reported and estimates were almost identical for both.	N
	7.3 ... different subgroups?	The only sub-groups are male and female and estimates were reported for both.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.16 Irani et al. 2019

Methods	Cross-sectional. ACT score used to measure asthma control
Participants	all consecutive asthma patients presenting to the outpatient allergy clinic at the Hotel-Dieu de France (HDF) University Hospital between January 1, 2014 and December 30,2016 (183 patients)
Interventions	Overweight/obesity
Outcomes	Asthma control measured by ACT score
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	There are many possible confounders of the effect of obesity.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	This was a cross-sectional study.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Multivariate logistic regression was used.	Y
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	The data was from a university hospital and participants were asked to fill in questionnaires for other data that was required	PY
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No post-intervention variables were adjusted for.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were selected based on their formal asthma diagnosis and were then assigned to treatment groups based on their BMI. This occurred at the start of intervention.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Start of follow-up and start of intervention were the same for all treatment groups	Y
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Intervention groups were clearly defined by their BMI	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Information used to define intervention groups was recorded before the start of the intervention.	Y
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	The classification of intervention status was done using BMI, which is not affected by knowledge of the outcome.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Participants did not switch between intervention groups, so any deviations from intended interventions would be expected.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Participants were selected based on their asthma status, so outcome data was available for all participants.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Intervention status was known for all participants.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	There was no mention of excluding participants due to missing data needed for the analysis.	PN
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	The outcome measure was determined by a medical professional and would not have been affected by BMI class.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Outcome assessors were aware of the intervention received for all participants.	Y
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods of outcome assessment were exactly the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	The outcome measurements were done by a medical professional and would not have been affected by BMI status.	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	There was only one outcome measurement.	N
	7.2 ... multiple analyses of the intervention- outcome relationship?	The results from each analysis were reported and discussed.	N
	7.3 ... different subgroups?	There were no subgroups.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

10.17 Viana de Jesus et al. 2017

Methods	Cross-sectional. Collected clinical, laboratory and anthropometric parameters, as well as pulmonary function test results and data regarding comorbidities. Participants also completed asthma control questionaires.
Participants	925 subjects with mild-to-moderate or severe asthma evaluated between 2013 and 2015
Interventions	Obesity (defined by BMI and waist circumference)
Outcomes	Number of neutrophils in peripheral blood, skin prick test results, spirometric values and uncontrolled asthma
Outcome measures	Differences between groups and associated p-values

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	There are many possible confounders of the effect of obesity.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	This is a cross-sectional study so there was no follow-up.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	The analysis method used was chi-squared tests and Kruskal-Walis tests, neither of which control for confounders.	N
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Not post-intervention variables were controlled for.	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Due to the nature of obesity, it was present before the beginning of the study and selection was based on asthma status.	Y
	2.2. If Y/PY to 2.1: Were the post- intervention variables that influenced selection likely to be associated with intervention?	There is evidence that obesity is associated with asthma prevalence.	PY
	2.3 If Y/PY to 2.2: Were the post- intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	Asthma prevalence is not affected by asthma severity but could possibly be affected by a cause of asthma severity.	PN
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Obesity onset is different for all participants.	N
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	Selection bias was not accounted for.	N
	Risk of bias judgement		Serious

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Obesity was well defined using body measurements.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Obesity onset occured before the beginning of the study.	N
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowledge of asthma severity would not affect classification of obesity status.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	This study was cross-sectional so there is no opportunity to deviate from intervention.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Outcome data was recorded for all the participants.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Intervention status was recorded for all participants.	N
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	No participants were excluded.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Asthma severity can not be influenced by knowledge of obesity status.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Obesity is an observable trait.	PY
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The methods were exactly the same across intervention groups	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	There were no systematic errors in measurement of outcomes.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	All results were reported.	N
	7.2 ... multiple analyses of the intervention- outcome relationship?	Only a single analysis was conducted.	N
	7.3 ... different subgroups?	There were no subgroups.	N.
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Serious

10.18 Lu et al. 2016

Methods	Cross-sectional. Participants completed cardiorespiratory fitness testing, body composition measurements, and respiratory questionnaires
Participants	4828 participants of 12-19 years of age. Data used came from the 1999–2004 National Health and Nutrition Examination Survey (NHANES)
Interventions	Overweight/obesity
Outcomes	History of asthma, current asthma, wheezing, asthma attacks, wheezing related to exercise, asthma-related ED visits, wheezing-related medical visits and wheezing-related missed days
Outcome measures	Odds Ratios

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study?	There are many possible confounders for the effect of obesity.	Y
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received?	This is a cross-sectional study so there was no follow-up.	N
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Regression model was adjusted for 6 confounders. There could possible be more confounders not accounted for.	PY
	1.5. Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Counfounding variables used in the study are easily measured and were measured using the same tools for all participants.	Y
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	No post-intervention variables were controlled for.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Participants were just all people who filled out the questionnaire. They were not selected based on any characteristics.	N
	2.4. Do start of follow-up and start of intervention coincide for most participants?	Obesity onset is different for all participants.	N
	2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	There is no mention of any techniques used to account for selection bias.	N
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in classification of interventions	3.1 Were intervention groups clearly defined?	Obesity was clearly defined using BMI percentiles adjusted for age.	Y
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	The relevant body measurements were taken in the survey but not at the start of obesity.	N
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Knowing about asthma status does not affect classification of obesity status.	N
	Risk of bias judgement		Low

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias due to deviations from intended interventions	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	There were no reported deviations from the intervention. Due to the nature of the intervention and cross-sectional design, deviations are not possible.	N
	Risk of bias judgement		Low

Bias domain	Signalling questions	Elaboration	Response options
Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	Only a very few participants were excluded because of lack of outcome data.	Y
	5.2 Were participants excluded due to missing data on intervention status?	Very few were excluded due to missing data on intervention status but some were.	Y
	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	For the section on the association between asthma and obesity participants were not excluded for missing data on any other variables.	N
	5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	Some participants were excluded due to missing data on intervention so it is not possible to determine this.	NI
	5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	This is not mentioned in the paper.	NI
	Risk of bias judgement		Moderate

Bias domain	Signalling questions	Elaboration	Response options
Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Asthma status is not affected by knowledge of obesity status.	N
	6.2 Were outcome assessors aware of the intervention received by study participants?	Possibly as obesity has a visible characteristic.	PY
	6.3 Were the methods of outcome assessment comparable across intervention groups?	The method was exactly the same across intervention groups.	Y
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	There were no reported systematic errors in measurement. However, the measurement was survey responses which leave room for error.	PY
	Risk of bias judgement		Moderate

Bias domain	**Signalling questions**	**Elaboration**	**Response options**
Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from ...		
	7.1. ... multiple outcome measurements within the outcome domain?	There were multiple outcome measurements but they measured different things and all were reported.	N
	7.2 ... multiple analyses of the intervention-outcome relationship?	There were not multiple analyses conducted of the intervention-outcome relationship	N
	7.3 ... different subgroups?	The two subgroups used were males and females but estimates for both were reported.	N
	Risk of bias judgement		Low
Overall bias	**Risk of bias judgement**		Moderate

Table 15: A table of key functions used in the *Mendelian Randomization* package.

Function	**Explanation**
mr_input	This function is required for inputting and formatting data for use in any of the estimation functions in the package.
mr_forest	This function draws a forest plot of causal estimates.
mr_funnel	This function draws a funnel plot of variant-specific causal estimates.
mr_loo	This function draws a forest plot of causal estimates omitting each variant in turn.

10.19 Key functions in the Mendelian Randomisation packages

Table 16: A table of key functions used in the *TwoSampleMR* package.

Function	**Explanation**
format_data	Reads in exposure data, then checks and organises columns for use with the package.
extract_outcome_data	Searches for the associations between each supplied SNP and the outcome of interest in the outcome-related study.
mr_heterogeneity	This function returns the heterogeneity statistics.
mr_pleiotropy_test	Performs an MR-Egger regression and returns the intercept values.
mr_singlesnp	Performs a 2 sample MR on each SNP individually.
mr_leaveoneout	Performs a leave-one-out sensitivity analysis.
mr_scatter_plot	Creates a scatter plot with lines showing the causal estimate for different MR tests.
mr_forest_plot	This function draws a forest plot of causal estimates.
mr_leaveoneout_plot	This function draws a forest plot of causal estimates omitting each variant in turn.
mr_funnel_plot	This function draws a funnel plot of variant-specific causal estimates.

Table 17: A table of key functions used in the *mrbayes* package.

Function	**Explanation**
mrinput_mr_format	Convert an object of class *MRInput* from the *MendelianRandomization* package to the *mrbayes mr_format* class.
mr_ivw_stan	Generates posterior samples using a Bayesian inverse variance weighted model with a choice of prior distributions fitted with RStan.
mr_egger_stan	Generates posterior samples using a Bayesian MR-Egger model with a choice of prior distributions fitted with RStan.