Dear Luisa/Ko,

Thanks for the time you gave me and put into examining my work. I really appreciate it! I have addressed each of your points in turn in the following pages. I have also corrected spelling mistakes throughout but have not highlighted these in this document. All these changes are also reflected on GitHub by looking at the following commit: <https://github.com/mattlee821/000_thesis/commit/3f89f4d82f3f47663d1fe4d93cd2f658db866a34>. Let me know if these changes are appropriate or if there is anything that needs clarifying.

Thanks!

Matt

External examiner comments

# In the discussion (and perhaps introduction), the assignment of the lipoprotein “metabolites” to specific subclasses (from XS to XL) should be discussed. Since this assignment is rather arbitrary, it could explain some of the overlap in the associated genetic instruments of similarly sized lipoproteins

I have added the following paragraph to the introduction in section 1.7 page 26:

Of particular consideration when using metabolomic data from Nightingale Health (as used in this thesis) is the way in which lipoprotein particles are identified and assigned to specific classes. Lipoproteins are grouped into five categories based on a density range: chylomicrons, VLDL, IDL, LDL, and HDL340,341. These categories broadly conform to the functions of lipoprotein molecules, with chylomicrons transporting lipids from the intestine to the bloodstream, VLDL, IDL, and LDL transporting lipids from the liver to other tissues, and HDL the primary component of reverse cholesterol transport341. The density of lipoproteins correlates well with their size, which is associated with disease outcomes342–344. However, there is some overlap in particle size across the density categories341. As such, two lipoproteins of the same density, and thus categorised within the same class, may be considerably different in size. Conversely, two lipoproteins of the same size may have substantially different densities and be assigned different classes. It may therefore be appropriate to consider the broad spectrum of lipoproteins when investigating associations rather than individual lipoprotein associations when contextualising results.

I have also made changes in the discussion chapter in the following paragraphs:

For analyses in Chapter 6 investigating the effect of metabolites on endometrial cancer, instruments were identified using the same strategy described above and applying the stringent LD r2 threshold used in additional analyses in Chapter 5. However, given the strong inter-correlated nature of metabolites and the fact many metabolites are products of one another, this instrumentation strategy may not be appropriate. This is further compounded by the way in which lipoproteins are identified and assigned to specific classes based on a density gradient. As a result, two lipoproteins of the same density, and thus categorised within the same class, may be considerably different in size. Conversely, two lipoproteins of the same size may have substantially different densities and be assigned different classes. This has implications for the identification of associated SNPs and studies have shown there is a considerable number of SNPs associated with more than one lipoprotein335–339. It is therefore possible that SNPs associated with any one lipoprotein may not conform to the third instrumental variable (IV) assumption - that the SNPs do not affect the outcome except through the exposure.

In Chapter 6, two metabolites (both lipoproteins) with evidence of an intermediary role on the effect of adiposity on endometrial cancer were identified. A total of 56 and 50 SNPs were associated at a genome-wide significance threshold of p-value < 5 x 10-8 and an LD r2 threshold of 0.001. Of these SNPs, 16 were shared across the two metabolites. It is likely both metabolites also share SNPs with many other metabolites, given that just over half of the 934 identified SNPs for the 53 metabolites investigated in Chapter 6 were associated with just one metabolite and the average number of SNPs associated with a metabolite was 49. This issue of SNP specificity in regards to metabolites has been looked at previously393. Findings suggested that SNP specificity was low (i.e., many shared SNPs) and, more often than not, the variance explained for any metabolite instrument was higher for an alternative metabolite. The non-specificity of instruments has two potential consequences - horizontal and vertical pleiotropy. However, distinguishing one from the other, with specific regard to metabolite instruments, is challenging634. In analyses here, a single instrumentation approach was used for metabolites and, given the likely shared genetic architecture across the tested metabolites, the presence of both horizontal and vertical pleiotropy cannot be ruled out. Alternative instrumentation approaches could have used a combined instrument or removed shared SNPs. In regards to the latter approach, future work should focus particularly on the use of colocalisation methods635 to identify shared genetic signals across related traits.

# The exclusion of two “beneficial” BF SNPs from the genetic BF instrument, after finding opposing results, should be discussed more extensively, since this pruning strategy seemed not part of the initial analysis plan.

Thank you for raising this point. The plan was always to include a number of different GWAS as sources for instruments of adiposity measures. At the time of these analyses, I was performing separate analyses of the effect of favourable adiposity and metabolites with colleagues in Exeter. This work brought to my attention the two SNPs within the BF GWAS. The SNPs were thus removed from the analyses presented in the thesis to provide an additional test of the instrument and clarify whether the instrument for BF was also capturing elements of a favourable adiposity phenotype. I have provided more discussion of these SNPs and the reasoning behind their exclusion within relevant sections.

In chapter 5, where these ‘favourable adiposity’ SNPs are first introduced in the methods (in the additional analyses section), I have made the following changes:

Whilst the main analyses employed the most common approach of identifying and using genetic variants as instruments in MR studies (i.e., taking exposure-related variants from the largest and most recent GWAS), there are a number of potential limitations to this approach within the context of this chapter. Firstly, genetic instrumental variables for BMI and WHR were obtained from studies using UK Biobank, which has shown evidence of latent population structure372,373. With regards to BF, instruments were obtained from a study which used different measures of BF, potentially leading to measurement heterogeneity. To further test the validity of the genetic variants used in the main analyses as instruments, a complementary set of genetic instrumental variables for each adiposity measure were obtained from alternative published summary statistics (Table of instruments available on GitHub). Additionally, two SNPs identified in the BF GWAS have also been associated with ‘favourable adiposity’47,565. Specifically, Yaghootkar et al., (2014 and 2016) identified favourable adiposity SNPs as those SNPs identified in a GWAS of insulin resistance which were also associated with increased BMI and triglycerides, decreased HDL, decreased risk of type 2 diabetes and coronary artery disease, and a more favourable blood pressure. The BF instrument may therefore not solely be a reflection of increased total body fat and may instead be capturing elements of the favourable adiposity phenotype. It was expected that removal of these SNPs would result in an instrument that was less heterogeneous.

In the results of chapter 5, additional analyses:

For BF, there was considerable similarity between the main analysis and the additional analysis when using SNPs from Lu et al., (2016) which did not include two SNPs previously identified as being associated with ‘favourable adiposity’ (Figure A.35 and A.36). More tests reached the multiple testing threshold when using the 5 SNPs from Lu et al., as opposed to the full 7 SNPs, this included associations with apolipoprotein-1, phenylalanine, tyrosine, glucose, and cholesterol esters in very large HDL. Given estimates were highly similar and CIs appeared tighter using the 5 SNP instrument, this may suggest that the 7 SNP instrument is less specific to the BF phenotype than the 5 SNP instrument. For the additional analysis, which used 76 SNPs from Hubel et al., (2016), MR results were considerably smaller and appeared to show conflicting directions of effect with that of the Lu et al., (2016) SNPs (both using 7 and 5 SNPs). CIs were much tighter and two metabolites (phenylalanine and glycoprotein acetyls) reached the multiple testing threshold. Correlation between the two Lu et al., (2016) SNP lists was high (Spearmans Rho = 0.93), however both the 5 (Spearmans Rho = -0.64) and 7 (Spearmans Rho = -0.52) SNP lists from Lu et al., (2016) showed weaker inverse correlations with the SNP list from Hubel et al., (2016).

In the discussion of chapter 5:

There is clearly complexity in the choice of instrument, especially with complex traits such as adiposity. This complexity is perhaps well demonstrated through the BF analysis. For BF, there was considerable difference in effect estimates compared to the BMI and WHR results, with a large proportion of estimates in the opposite direction to those of BMI and WHR. This is counter-intuitive given the strong correlation between BMI, WHR, and BF, and the consistent results obtained in observational analyses in Chapter 4 between BMI, WHR, and BF. Removal of two SNPs previously associated with ‘favourable adiposity’47,565 resulted in a global tightening of CIs and a number of effect estimates changing direction to be more consistent with observational BF results and results for BMI and WHR here. A number of these effects subsequently reached the multiple testing threshold. However, the majority of MR results remained opposite to that of BMI and WHR. Though there was little difference in the F- statistics for either instrument, global tightening of CIs may suggest an instrument that is more specific to BF biology (less heterogeneous) after removal of the two ‘favourable adiposity’ SNPs. In post-hoc analysis investigating the directional inconsistency between BMI and WHR and BF, effect estimates from a single-SNP MR using rs1558902 to instrument BMI and BF resulted in highly consistent directions of effect across all Kettunen metabolites (data not shown). Results differed only in their effect size and standard error. This difference was in line with the difference in the SNP beta from the BMI (0.082) and BF (0.051) GWAS. Leave-one-out analysis for BF instrumented using the 7 genetic variants identified in Lu et al., (2016) did not indicate a single-SNP that could be driving a pleiotropic association. However, median effect estimates for rs6857 (rs6857 is associated with *NECTIN2* and has previously been associated with a number of diseases including Alzheimer’s disease591) were much larger than those for other SNPs, both with and without exclusion of the two ‘favourable adiposity’ SNPs. In many cases, rs6857 did not span the null. It is possible that tests for pleiotropy were underpowered however. The unexpected results for BF instrumented using the Lu et al., (2016) genetic variants may be due to a variety of reasons, not least measurement error, sample size differences between BF (up-to 89,297), BMI (up-to 795,624), and WHR (up-to 697,702), and the variance explained by the respective instruments: BF = 0.416%, WHR = 3%, BMI = 6%. The complexity of instrumentation is discussed further in the discussion Chapter 7 as this is also relevant to Chapter 6.

In the discussion chapter, I have then discussed the use of the 5 SNP instrument further in the “Methodology, instrumentation, and assumptions” section with the inclusion of the following paragraph:

A particular consideration with analyses in this thesis was the use of multiple instrument lists obtained from different GWAS. Ostensibly this was to test for the potential effects of population structure in BMI and WHR instruments obtained from GWAS, which either did or did not include individuals from UK Biobank, and of measurement heterogeneity in the BF GWAS, where BF was measured using two different methods. In addition, two SNPs within the BF instrument had recently been associated with ‘favourable adiposity’47,565. Specifically, these SNPs had been associated with both increased BF and reduced risk of type 2 diabetes, hypertension, and heart disease, as well as more favourable blood pressure565. As such, the BF instrument may not just be estimating an increase in total body fat mass but may also be capturing a paradoxical favourable metabolic profile with increased adiposity, which may thus produce a less specific estimate of increased body fat. In order to test whether these ‘favourable adiposity’ SNPs resulted in a less specific instrument for BF, they were excluded in additional analyses in Chapter 5. Results were highly consistent across the two instruments, however CIs appeared tighter across all metabolites when using the instrument which did not include the ‘favourable adiposity’ associated SNPs. Given there was little difference in F-statistics for the two instruments the global tightening of CIs may have been a result of a more specific instrument. Although MR results from this 5 SNP instrument were more consistent with observational results, further work to characterize and establish the relationship between the 7 BF SNPs and BF is needed. Additionally, assigning these SNPs as ‘favourable adiposity’ SNPs is likely a simplification of their biological function. An alternative approach could be to obtain instruments from a GWAS of localised body fat (e.g., trunk fat, arm fat, visceral adipose tissue). In this approach, SNPs associated with a specific compartment (i.e., visceral adipose tissue) would be used to instrument a more homogeneous body fat measure.

# P97 Misspelling: covarialbes (p97 and following)

Done

# P62 Numbers for type 2 diabetes (first paragraph) and asthma (second paragraph) do not match table 2.7

There is no table 2.7, I believe you mean Figure 2.7. The numbers in the text now align with the correct numbers presented in the figure.

# P114 Derived measures figure number missing (last paragraph)

Done

# P121 Positive/negative association reversed (last paragraph)

Done

# Outline an P122: Misspelling ALSAPC

Done

Internal examiners comments

# Consider using the first person more (you did the work). Particularly in Chapter 3 and in describing results of collaborative work (also in Ch6 and elsewhere where you say you have collaborated with others

This is a good point, thank you. Whilst I agree with this comment, I also wish to keep the same narrative throughout. Therefore, I have not used the first person in these chapters but I have added clarity in the contributions chapter that the presented work is mine:

All of the work in Chapters 1, 4, 5, and 6 is solely mine. Work presented in Chapters 2, 3, and 6 involved a number of collaborations, however these projects were led by myself and the presentation of this within the Chapters is solely mine. Specifically, I lead all of the work in Chapter 2, which was conducted in collaboration with Charlie Hatcher, Luke A McGuinness, Nancy McBride, Thomas Battram, Si Fang, Wenxin Wan, and Kaitlin H Wade who all con- tributed to data extraction. I also performed data extraction and, in collaboration with Charlie Hatcher, checked all extracted data for all included studies. I performed all analyses in Chapter 2. In Chapter 3, work was conducted in collaboration with Osama Mahmoud and Luke A McGuinness. I worked with Osama to develop the code that would form the original draft of the visualisation tool, EpiViz. Luke McGuinness helped with aspects of the Shiny application. The following colleagues provided feedback in the development of EpiViz: Caroline Bull, Charlie Hatcher, Kurt Taylor, Nancy Mcbride, Neil Goulding, and Steph Suddell. Work from Chapter 3 is published open source on GitHub and is presented for the first time in Bos et al. (2020)1. Vanessa Tan and Caroline Bull shared multivariable Mendelian randomization (MVMR) scripts with me, which I then used for reference in my analyses in Chapter 6. Eleanor Sanderson provided helpful guidance and feedback on the interpretation of MVMR results in Chapter 6.

# Start each Chapter with a clear description of what work you produced, added to, edited, contributed to etc (a detailed contribution statement)

I have made it clearer (in response to the above point) within the contributions section at the start of the thesis what work is solely mine and where I collaborated with others. I have included the same information at the start of each under the chapter, for example:

I performed all of the work in this chapter.

# When discussing MR more in details beyond chapter 1, define horizontal pleiotropy and contextualise to the case of metabolites as exposures or intermediates (‘upstream’ Vs ‘downstream’ pleiotropy, independent pathways, etc).

This is a really good point and draws on the first point made by the external examiner about the assignment of lipids. As you will see from my response to their point, I have included additional paragraphs to the discussion chapter which highlights the difficulty that the assignment of lipids poses to instrumentation. To address your point here further, I have included the following paragraphs expanding on pleiotropy in chapters 5 and 6 to contextualise the results. I believe addressing the first point from the external examiner and these additional paragraphs address your concerns here.

In chapter 5, I have added the following paragraph at the top of the discussion to contextualise subsequent discussion:

It is important when interpreting the results presented here to acknowledge the potential effects of pleiotropy in these analyses. As discussed in Chapter 1, the effects of pleiotropy in an MR context can either be vertical (on the causal pathway from exposure to outcome) or horizontal (on an alternative causal pathway to the outcome). Importantly, a change in any one metabolite does not occur in isolation; metabolic pathways mean that many metabolites will be up- or down-stream of a metabolite under investigation. An additional consideration regarding lipids is the way in which lipids are identified using a density gradient. This means that highly similar lipids (e.g., in terms of size) can be assigned to different classes, while dissimilar lipids (e.g., different sizes) can be assigned to the same class. This consequently impacts on the observed genetic architecture of the lipids which feeds into subsequent MR analyses. Taken together, it may be difficult to ascribe a direction to any observed pleiotropy. Sensitivity analyses used here, such as MR-Egger, can provide an estimate as to whether horizontal pleiotropy is present in a test and results should be considered in regard to evidence of the presence of horizontal pleiotropy for a majority of MR tests.

In chapter 6, I have included a similar paragraph to the top of the discussion to contextualise subsequent discussion:

It is important when interpreting the results presented here to acknowledge the potential effects of pleiotropy in these analyses. As discussed in Chapter 1 and 5, the effects of pleiotropy in an MR context can either be vertical (on the causal pathway from exposure to outcome) or horizontal (on an alternative causal pathway to the outcome). In the context of metabolites, ascribing a direction to any observed pleiotropy can be difficult given the highly intercorrelated nature of metabolites and, specifically for lipids, the way in which they are identified and assigned. As a result, the common shared genetic architecture of many metabolites has implications for their use in MR analyses as an exposure, mediator, or outcome. For an instrument to be specific, the SNPs should only be associated with that one phenotype under investigation. However, in the case of metabolites, many SNPs associated with any one metabolite are also found to be associated with many related metabolites335,336,339. Additionally, the proportion of phenotypic variance explained by an instrument for one metabolite can be greater for another metabolite393. As such, instruments for any one metabolite will not only capture variance in that metabolite but related metabolites that may both be on the causal pathway and on alternative pathways. Here, it is assumed that the causal pathway is from adiposity to metabolites to endometrial cancer. Results should thus be interpreted with this and evidence from methods sensitive to pleiotropy which indicated presence of pleiotropy in mind. In particular, in MVMR analyses there was evidence of horizontal pleiotropy for analyses involving overall endometrial cancer and endometrioid cancer.

# Chapter 1

## First paragraph – unclear what this means in this sentence “Whilst ethnicity, sex, and age specific”

I have clarified this in the paragraph:

While BMI classifications of underweight, normal weight, overweight, and obese are ethnicity-, sex-, and age-specific, the international standards set by the World Health Organisation (WHO)70,71 estimate a normal weight classification at a population level to be a BMI of 18.5–24.9 kg/m2, with an underweight class below this.

## Second paragraph- please elaborate on this, eg include reference to studies comparing BMI to other measures of adiposity “The ability of BMI to categorise individuals as at risk for particular conditions in an efficient manner is a major benefit over other more costly and time consuming measures of adiposity”

I have expanded the paragraph to include this information:

Given the ease of its measurement, BMI is the most predominant marker of adiposity in large-scale epidemiological studies. Thus, the relationships between BMI and many of the most abundant diseases and causes of death worldwide are more comprehensively characterised compared to other measures such as WHR and BF. This includes all cause mortality, hypertension, type 2 diabetes, stroke, respiratory problems, many cancers, and more72,73. In a large review and assessment74 in which adiposity measures were categorised as assessing total body adiposity (e.g., BMI), assessing distribution of body fat (e.g., WHR), assessing body composition (e.g., BF), and assessing ectopic fat, BMI was recommended as the primary tool for measuring adiposity in populations especially given its simplicity. They further recommended that, given the heterogeneity in individual body fatness observed at a given BMI, the assessment of body composition alongside distribution using simple clinical tools would be most beneficial in identifying individuals with excess visceral and liver fat.

## End of subchapter refers to composite measure of adiposity but not clear what this refers to.

 I have clarified this:

Given the individual limitations of BMI, WHR, and BF measurements, a complimentary assessment of adiposity, using all three measures in turn and comparing estimates, may be beneficial when investigating associations with disease95,100. This is especially important as genetic analyses point to key differences between the underlying biology of adiposity measures34 that may be relevant to the relationship between adiposity and disease.

## p27- please correct the definition of confounder, as it is not “Measured or unmeasured factors that are associated with the exposure and the outcome, confounders, can bias results”

I have amended this:

Confounders (i.e., common causes of the exposure and outcome that can be measured or unmeasured) can bias results.

## Fig 2: Z should be a genetic variant if it is MR, or an IV if it is a more generic ‘Instrumental Variable approach’

Done

## P29- “that could effect the environment” should read “affect”.

Done

## P30- improve the accuracy of the definition of population structure. Especially important as you discuss the implication of failures to adequately adjust for population structure later on p32 (and what is the relevance for this thesis?)

This is a good point. I have improved the definition of population structure in the paragraph:

Population structure is a result of subgroups within a population existing due to differences in phenotypes, allele frequencies, and haplotype (linkage disequilibrium) structure. This would also violate the second MR assumption as the association between the IV and risk factor could be confounded by the subgroups. In MR analyses it is assumed that latent structure is accounted for in the GWAS in which the IVs are discovered372. As the sample sizes of GWAS has increased, the potential for subtle effects of population structure have been observed372,373. Though one can restrict analyses to homogeneous groups, use principal components, and perform within family studies to examine and mitigate the effects of population stratification, biases (e.g. sampling bias) may still remain370.

## p32- a more explicit discussion of horizontal pleiotropy as a cause of the exclusion restriction assumption is needed, especially since the following paragraph mentions vertical pleiotropy

I have expanded the paragraph as:

When MR was first described, traits were instrumented mostly using a small number of well characterised SNPs. These SNPs would explain a small amount of trait variance, but the underlying biology was understood. More recently, GWAS are, and have been, able to identify large numbers of SNPs which explain ever larger proportions of trait variance. With this added power however comes a more complex instrument with many potential biological mechanisms linking SNPs to the trait. In an omnigenic model, variance in a trait of interest is not solely a result of directly related genes (core- genes). Rather, all genes expressed in relevant cell types have an effect, however small, on the trait of interest29. These peripheral genes, which have no obvious direct link to the trait of interest, are mostly in non-coding regions with regulatory functions67. Given this, the fact that variants associated with complex traits are dispersed widely across the genome67, and the difficulty in assigning a link between any particular SNP and an individual gene68, variants associated with complex traits likely implicate many genes with the trait. Because many of these will be peripheral genes (not core genes), they will ultimately affect other traits, which in an MR context may include the outcome and thus violate the exclusion restriction assumption (commonly termed the no horizontal pleiotropy assumption). For example, a GWAS of BMI identifies SNPs associated with increased BMI. A number of the genes associated with these SNPs are expressed exclusively in the brain and are associated with behavioural changes. These behavioural changes are primarily to do with satiety, but some are associated with increased risk-taking behaviours such as smoking. As such, an association may now be induced between BMI and lung cancer given there is an association between some of the BMI SNPs and smoking which is a causal risk factor for lung cancer – it is assumed that smoking is not caused by BMI. In this hypothetical scenario, the pleiotropic SNPs are known, can be excluded from analyses, and the true causal effect can be estimated.

## last paragraph needs rewording – from “An alternative approach” this needs to be a new paragraph. The approach mentioned is also known as ‘meet in the middle’ – see appropriate references

I have moved the first sentence from this paragraph to the end of the previous paragraph:

MVMR is a form of mediation analysis which allows for the causal effects of multiple exposures on an outcome to be estimated382 (Figure 1.4). The effect of each exposure is estimated conditional on the other exposures and thus provides a direct estimate of the effect. Figure 1.4 shows a simplified MVMR model with two exposures (X1 and X2). The indirect effect is estimated by subtraction of the direct effect from the total effect. The total effect is calculated using univariable MR. As with two-step MR, no interaction between exposure and intermediate is assumed. Though a new approach, and still subject to the same assumptions as with two-step and univariable MR, MVMR has shown promise in elucidating underlying aetiology of complex traits387–390. Though two-step MR was devised with epigenetic mechanisms in mind381 and MVMR has shown promise investigating metabolic intermediates389, their application to large omic data sets is yet to be shown.

I have then expanded the final paragraph as:

An alternative approach is to look for overlapping signals (commonly known as a meet-in-the-middle approach)391. Generally, this is considered in three steps: 1) association between exposure and outcome; 2) association between candidate intermediate of the exposure and outcome; 3) association between outcome and candidate intermediate of the exposure. Evidence for an association is strengthened if observed across all three steps392. In this regard, the effect of the exposure on the candidate intermediate and the effect of the candidate intermediate on the outcome are ranked in terms of their effects and an intermediate is considered if it ranks highly in both analyses393.

# Chapter 2

## Narrative review – when summarizing results per outcome category, start with bottom line eg “The effect of adiposity appears far reaching in regards to metabolic traits. This effect is generally to increase levels of traits that are themselves associated with poor health outcomes.” for the metabolites section, or make the bottom line more apparent eg by starting a new paragraph.

This is a good point and I can see some of these summary points are not clear. I have made each summary point its own paragraph at the end of each section as you suggested.

# Chapter 5

## Draw more emphasis on the remarkable consistency between the selected MR and observational results (Fig p176 – no figure number). How do you interpret this (they are not independent tests)? What does this tell us in terms of the relationship between the different metabolites, and whether our priors are confirmed in terms of direction of association?

I have fixed the missing figure number. I have then split the paragraph at the top of the discussion in two and expanded on the second paragraph:

In this chapter, the influence of adiposity on the metabolic profile is demonstrated in an MR framework. The use of MR allowed the interrogation of causality of various measures of adiposity on the metabolic profile, while accounting for limitations in observational analyses (discussed in Chapter 1 and 4). Data on adiposity measures were available for: BMI from up to 795,624 individuals of European ancestries from GIANT53, WHR from up to 697,702 individuals of European ancestries from GIANT54, BF from up to 89,297 individuals European ancestries from Lu et al., (2016)51. Two parallel MR analyses of 123 NMR derived metabolites measured in up-to 24,925 individuals of European ancestries from Kettunen et al., (2016)336 and 230 NMR derived metabolites measured in up-to 40,905 individuals of European ancestries from INTERVAL (unpublished) were conducted. Meta-analysis of 110 metabolites measured in both the Kettunen and INTERVAL datasets and comparison with observational analyses from Chapter 4 identified 54 associations between adiposity and metabolite measures that were consistent in direction of effect across MR and observational analyses and passed multiple testing thresholds for one (BF) or both (BMI and WHR) analyses.

Consistency in estimates across exposures and observational and MR analyses for the 54 associated metabolites highlights the importance of overall body composition and deposition of adipose tissue in the relationship between adiposity and the metabolome. Positive associations were found for metabolites in VLDL (small, very small, medium, large, and very large), as well as aromatic and branched chain amino acid subclasses. While negative associations were found for HDL (medium, large and very large) subclasses. It is important to note that many metabolites are not independent, that is they share the same class and subclass. As such, consistency in the direction of effect across metabolites within a subclass may be expected given these metabolites are highly interrelated. That being said, there is evidence for a difference in direction of effect across the HDL subclasses with small HDL metabolites positively associated with BMI, WHR, and BF in comparison to the negative association observed for medium, large, and very large HDL. This could suggest that small HDL metabolites are distinct from other HDL subclass metabolites, or that the effect of adiposity is differential depending on particle size.

# Chapter 7

## Mention alternative study designs that could help solve some of the more challenging research questions with public health relevance eg accumulation of adipose tissue over different time-frames, ‘reversibility’ of a detrimental metabolic profile consequent to fat accumulation, etc

In the future work section I have added a paragraph with information and ideas for alternative study designs:

In addition, investigations will benefit from alternative study designs, which are able to address more specific challenges such as weight change and whether the effects of adiposity are reversible. With regards to weight change, a recent review attempted to highlight the effect of weight gain and weight loss on the metabolome667. The vast majority of studies included in the review looked at the effects of weight gain and found there to be a generally increasing effect across many metabolite subclasses including lipids (e.g., VLDL, LDL, and large HDL) and amino acids. The few studies included which looked at weight loss were focused on the effect of specific diets and the insulin related changes of these diets. Recently, data has become available from a weight gain and weight loss study (Glasgow Visceral & Ectopic Fat With Weight Gain in South Asians), which aims to investigate whether there are differences in fat storage and metabolic risk factors throughout the weight gain and loss phases. Studies that look at metabolite changes due to weight gain and loss will be important in understanding whether the effects of adiposity, that is weight gain, are reversible over the long term. Identifying if and what effects of adiposity persist after sustained weight loss may help to target interventions and prolong that weight loss. For example, there is some evidence that diet-induced weight loss is unsuccessful in the long term due to an increase in ghrelin over time668. It may be possible to look at the effects of sustained weight loss on the metabolome in ALSPAC and UK Biobank as, in both studies, repeat measures were obtained for metabolomic analysis. However, there may be insufficient samples to investigate disease outcomes. Instead, a meet-in-the-middle approach could be used. In the first instance the effects of weight loss are investigated in a large study like UK Biobank. This can then be followed up with analyses using data from clinical studies, for example of people in remission from type 2 diabetes. Metabolites identified in both studies are then ranked and may be considered as candidate intermediates in the relationship between sustained weight loss and disease outcome.