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**Body mass index, obesity and adiposity: has epidemiology started to break down causal contributions to health and disease?**

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1  
2 **TITLE: Body mass index: has epidemiology started to break down causal contributions to health and**  
3 **disease?**

4  
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## Study Importance Questions

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4 1. What major reviews have already been published on this subject?  
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7 a) Loos, R.J.F. (2012) Genetic determinants of common obesity and their value in prediction,  
8 *Best Practice & Research Clinical Endocrinology & Metabolism*, 26(2): 211-226.

9 This paper reviews the discovery of loci associated with obesity-related traits, and  
10 subsequently focuses on the body mass index (BMI) loci in particular to explore whether  
11 there is sufficient evidence for these loci to be used as clinical predictors. It does not  
12 consider use of loci in causal analyses.  
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15 b) Burgess, S. *et al.* (2015) Mendelian randomization: where are we now and where are we  
16 going? *International Journal of Epidemiology*, 44(2): 378-388.

17 This editorial considers developments in the methodology and application of Mendelian  
18 randomization to study causal mechanisms in health and disease over the past decade. It  
19 does not consider adiposity, BMI or obesity specifically.  
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23 2. What does our study add?  
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25 Progress in the field of applied genetic epidemiology and in particular in the application of  
26 Mendelian randomisation has been rapid in the last few years, driven largely by developments in a  
27 variety of omics technologies. This review provides a reflection on what has been achieved so far  
28 in dissecting the causal relationship between body mass index (BMI) and disease and gives  
29 comment on the likely future directions of this field. We also present a sobering discussion of the  
30 potential limitations of these approaches which are becoming commonplace in the field of  
31 complex trait analysis, especially for BMI in light of large-scale consortium science.  
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**Abstract**

*Objective:* To review progress in understanding the methods and results concerning the causal contribution of body mass index to health and disease.

*Method:* In the context of conventional evidence focused on the relationship between BMI and health, we consider current literature on the the common, population based, genetic contribution to body mass index BMI and how this has fed into the developing field of applied epidemiology.

*Results:* Technological and analytical developments have driven considerable success in identifying genetic variants relevant to BMI. This has enabled the implementation of Mendelian randomization to address questions of causality. The product of this work has been the implication of BMI as a causal agent in a host of health outcomes. Further breakdown of causal pathways by integration with other omics technologies promises to deliver additional benefit.

*Conclusion:* Considerable progress has been made, though gaps remain in our understanding of BMI as a risk factor for health and disease. Whilst promising, applied genetic epidemiology should be considered alongside alternative methods for assessing the impact of BMI on health and in light of potential limitations which relate to inappropriate or nonspecific measures of obesity and the improper use of genetic instruments.

**Introduction**

The pandemic status of high BMI (obesity) has been attributed to the rise of an “obesogenic” environment which tips the balance between energy intake and energy expenditure, driving individuals towards

1 increased adiposity along environmentally determined lines (1, 2). Despite this it is important to realise  
2 that, within the same environment, not all individuals become overweight or obese and those that do have  
3 differential disease risk. In reality, a complex interplay of both genetic and environmental factors must be  
4 considered in order to better understand BMI and why it appears to have such a great health impact.  
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10 Focusing on BMI specifically, whilst it is absolutely clear that there are strong and replicable associations  
11 between this risk factor and health, the interpretation of existing associations is not straightforward. In  
12 reality, a complex interplay of both genetic and environmental factors must be considered in order to  
13 better understand BMI as a phenotypic proxy for adiposity, why it appears to have such a great health  
14 impact and how this impact might be mitigated in future both at the individual and the population level.  
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23 The underlying aetiology of relationships between BMI and health outcomes is clearly complex and likely  
24 to be heterogeneous across differing populations, apparently healthy individuals and those with disease. It  
25 is perhaps not surprising that efforts to counter the impact of ~2.3billion overweight, >700million obese  
26 and ~\$100billion per annum care bill (including targeted dietary intervention (3), weight loss programmes  
27 (4, 5) and pharmaceutical interventions (6, 7)) have failed to deliver lasting reductions in BMI (>2yr) at least  
28 at the level of the population. Currently, the only effective intervention for weight reduction is bariatric  
29 surgery, which is costly, not favourable for the treatment of moderate obesity (8).  
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40 Questions therefore remain as to why we continued to focus on BMI when we struggle to understand it as  
41 a measurement and fail to control or augment it at a policy or population level? More so, if we are content  
42 that the ease of BMI measurement is a justification for continued use, how might we gain insight into how  
43 and why BMI appears to be causally related to disease? In the context of conventional evidence focused on  
44 the relationship between BMI and health, this review aims to consider the current literature around the  
45 common, population based, genetic contribution to BMI/adiposity and how this has fed into the  
46 developing field of applied epidemiology in an effort to assess if and how the metric  $\text{kg/m}^2$  exerts a causal  
47 effect on health. In doing this, we will discuss the complications of measurement, complex genetic  
48 aetiology and idiosyncrasy of human phenotyping (and its effect on analysis and inference) before  
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1 attempting to suggest likely future moves for BMI research.  
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### 6 **Conventional approaches to the analysis of BMI and health** 7

8 More than 1.9 billion adults, 18 years and older, were overweight with over 600 million obese in 2014.  
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10 This represents the worldwide prevalence of obesity more than doubling between 1980 and 2014 and the  
11 consequences of this are put into morbid focus when one is reminded that raised BMI is a substantial risk  
12 factor for disease cardiovascular disease (which were the world leading cause of death in 2012), diabetes,  
13 musculoskeletal disorders and some cancers (endometrial, breast, and colon) (World Health Organisation,  
14 2015). The evidence for these relationships comes from a variety of sources, but importantly the relative  
15 simplicity of height and weight measurement has allowed for the formation and analysis of substantial  
16 BMI related data sets focused on these relationships.  
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28 Examples of this include the Prospective Studies Consortium (able to assess observational relationships  
29 between baseline BMI and mortality in a collection of 57 studies delivering 894,576 participants mostly  
30 from Western Europe and North America(9) and an equally well sized initiative in Asian population based  
31 samples (including more than 1.14 million people recruited in 20 cohorts in Asia(10)) which have been able  
32 to give estimates as to the likely contribution of BMI variation to the risk of death and specific disease  
33 outcomes. Away from population specific differences hinting at the potential importance of body  
34 composition in BMI related effects, the relationship between BMI and mortality (with a marked  
35 cardiovascular component) is broadly consistent. Whilst not proven to be fully causal, these studies  
36 present a compelling illustration of these relationships.  
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49 This type of work has not been limited to the collection of semi-focused, large-scale investigations of  
50 mortality and common disease outcome, but also has been undertaken in a manner targeting specific  
51 disease outcomes. For example, a detailed examination of UK Clinical Practice Research Datalink (CPRD,  
52 www.cprd.com) was able to characterize the observational associations between BMI and cancer risk for  
53 the 22 most common cancer sites seen in UK medical record data(11). In this work, more than 5 million  
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1 individual records reporting over 160000 cancers were investigated yielding evidence of association  
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3 between BMI and 17 of 22 disease sub-types. Outside of likely confounding events driven by smoking,  
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5 compelling association between BMI and disease risk are evident for cancers of the uterus, kidney, thyroid  
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7 and leukaemia with more complex association signatures seen for liver, colon, ovarian breast cancers and  
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9 together add to the growing range of non-specific disease risk alterations that appear linked to population  
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11 based fluctuations in BMI.  
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17 Outside the realm of observational epidemiology, interventional studies in the form of randomized control  
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19 trials (RCTs) have of course been applied. The most commonly evaluated interventions for BMI involve  
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21 modifications to diet and/or physical activity levels as implemented in both children (12, 13) and adults (5,  
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23 14, 15, 16, 17). There are also RCT that have tested the efficacy of pharmacological interventions, most  
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25 often alongside behavioural changes with the most commonly tested agent being Orlistat (4, 18, 19) .  
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27 However, the potential for pharmacological intervention is somewhat limited due to a lack of suitable  
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29 drugs with favourable properties (20). Whilst most, although not all (12, 13), behavioural and/or  
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31 pharmacological interventions result in a reduction in adiposity (as assessed by BMI or body weight), a  
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33 major limitation of these studies with respect to inferring causality between BMI and health, is the lack of  
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35 long-term follow-up, with a 12-month endpoint being typical. Therefore, the conversion of this reduction  
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37 in BMI to a reduced incidence of disease later in life is not well evidenced. Indeed, apparently beneficial  
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39 changes in cardiovascular risk factors, such as lipid profile and blood pressure, have been used to bolster  
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41 conclusions regarding health benefits despite results from at least one longer-term trial suggesting that  
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43 the assumed link between these intermediates and cardiovascular mortality may not be valid (14).  
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49 In contrast to most behavioural intervention studies, RCTs of surgical intervention have had longer follow-  
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51 up periods allowing a more direct assessment of the impact of weight reduction on mortality. The long-  
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53 term health impact results of RCTs for surgical intervention have been mixed and whilst there is evidence  
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55 of a reduction in mortality following surgery (21, 22, 23), concerns have been raised around the potential  
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57 for differences between surgical cases and untreated controls to complicate analyses (22). There is also no  
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1 assurance that the effects seen after these procedures is directly related to BMI/weight reduction, with  
2 short term impact of surgery being marked and arguably independent of weight(24). Furthermore, the  
3 cost-effectiveness of surgery depends on the patient's level of obesity on admittance and the relative  
4 improvement in quality of life and health achieved subsequently (25).  
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12 Taken together, whilst there is a deep literature focused on the examination of associations between BMI  
13 and common health outcomes within both observational and intervention designs, these approaches  
14 remain limited in their ability to assess the causal contribution of BMI to disease. Observational studies  
15 have been undertaken at scale, but retain the conventional limitations to inference in confounding, bias  
16 and reverse causation and although trials of BMI intervention are conceptually more inceptive, limitations  
17 to the interventions themselves and the ability to alter BMI hamper the interpretation of long term health  
18 implications.  
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### 30 **Genetic contributions to BMI**

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32 Common form obesity, assessed simply by BMI level and which does not segregate in families, has a  
33 multifactorial basis. Individuals may carry any number of common genetic variants which contribute to  
34 variation in BMI at the level of the population, but most of these exert only small effects on adiposity.  
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36 Genomewide association studies (GWAS) employ a hypothesis-free approach to identify variants  
37 consistently associated with complex traits (26) and use genotyping chips with the ability to score  
38 hundreds of thousand to millions of single nucleotide polymorphisms (SNPs) positioned across the entire  
39 genome. This approach has revolutionised the search for genetic associations and the interrogation of the  
40 common disease/common variant hypothesis specifically (27) and in the case of BMI, the first real progress  
41 in the application of GWAS approaches came with a study of just under 40,000 participants and from an  
42 initial search for type 2 diabetes loci (28). This work identified a locus with common variants reliably  
43 associated with BMI where carriers of two copies of the minor allele at *FTO*rs9939609 were on average 3kg  
44 heavier than the major allele carriers (29). Immediately after this first wave of GWAS analyses, it was  
45 acknowledged that substantially larger sample sizes, greater genomic coverage through advanced  
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1 reference panel use and imputation (30) and more rigorous discovery and replication phases through  
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3 extensive consortia derived meta-analysis were needed to fully explore the common genetic contribution  
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5 to complex traits like BMI (31, 32). The most recent of these involves 125 independent cohort studies and  
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7 totalling nearly 340,000 participants and has brought the list of confirmed associated genetic variants to  
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9 97 (**Figure 1**) (33).

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14 Despite the recent success in identifying and verifying almost 100 loci with confirmed BMI association,  
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16 together they only explain in the region of 2.7% of the phenotypic variance in BMI (33). Even with the  
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18 addition of these new associated genetic variants, it is evident that although each new (bigger) GWMA  
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20 offers new biological insight through novel gene discovery, the newly discovered associations are the  
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22 product of larger studies and not larger effects (**Figure 2**). Saving the scaling up of population based  
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24 sequencing initiatives with the capacity to score rare variants(34), the next steps are therefore to make use  
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26 of the variants we have to try to understand the effects of BMI. Importantly even small genetic effects are  
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28 potentially useful for this in the correct conditions and the development of MR has given utility to the “so  
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30 what” gene variant associations GWAS is efficient at capturing.  
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### 36 **Applied genetic epidemiology and Mendelian randomisation**

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38 Developments in the genetics of obesity have opened up a new avenue of investigation to researchers  
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40 interested in dissecting the relationship between BMI and health – Mendelian randomisation (MR). In  
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42 contrast to direct measurement, germline genotypes reliably associated with risk factors can act as proxy  
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44 measurements for risk factors offering several advantages: Genotypes are relatively easy to measure, are  
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46 stable through time, are largely immutable and are not correlated with confounding factors as a result of  
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48 the mechanisms of Mendelian inheritance(35, 36). An alternative approach to the analysis of BMI is  
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50 therefore to use genetic predictors to act as proxies of the feature (or exposure) one is concerned with in  
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52 order to help investigate causality(37, 38, 39)(**Figure 3A&B**). In MR, genetic variation fulfils the role of an  
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54 instrumental variable (40) where the presence of variance in BMI explained by genotype is orthogonal to  
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56 confounding factors and where genotype is assumed only to exert effect on health outcome through BMI.  
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1 Whilst these assumptions are clearly open to challenge (through pleiotropy and other phenomena  
2 discussed at the end of this review), this approach provides an important contribution to the weight of  
3 evidence that may exist around a given epidemiological association. It is important to note that applied  
4 genetic epidemiology and MR is just one approach to the assessment of causality outside of RCTs(41).  
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12 The first application of MR to BMI followed rapidly on from the discovery of *FTO*(rs9939609) and examined  
13 10 metabolic traits. Authors of this study concluded that the *FTO* genotype was associated with metabolic  
14 traits to an extent entirely consistent with its effect on BMI although power limitations meant causal  
15 relationships could only be confirmed for fasting insulin, glucose, triglycerides and lower high-density  
16 lipoprotein cholesterol (HDL-C) (42). By exploiting the ability of genetic variants to model lifetime  
17 exposure, researchers have also been able to explore the potential long-term effects of increased adiposity  
18 on health. To date, MR studies using BMI-associated variants have provided evidence of a causal effect of  
19 greater adiposity on a number of indicators of reduced cardiovascular health, including increased blood  
20 pressure (43), increased fasting glucose and insulin (44), decreased HDL-C (44) and increased systemic  
21 inflammation (45). Causal inference with respect to complex diseases is challenging however, a causal role  
22 for increased adiposity has been evidenced for type 2 diabetes T2D) (44) and ischaemic heart disease (IHD)  
23 (46). There are also examples of MR being applied to outcomes and traits beyond the classical  
24 cardiometabolic outcomes, including mental health (47, 48), childhood asthma (49), bone mass in  
25 childhood (50), uric acid (51), cancer (52) and trans-generational effects such as foetal over nutrition (40).  
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45 As well as these simple investigations of the causal impact of BMI on health-related factors, bidirectional  
46 assessments (39) have also been undertaken (**Figure 3C**). For example, exploring the relationship between  
47 the acute phase reactant C-reactive protein (CRP) and BMI, bidirectional MR has been used to exploit  
48 variation at independent BMI and CRP associated variants to evaluate whether BMI had a causal effect on  
49 CRP and simultaneously variation at the *CRP* loci to assess whether CRP had a causal effect on BMI (53).  
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55 This work has provided evidence implicating BMI as a causal agent in inflammation and asserting  
56 directionality in an otherwise unclear network of complex phenotypes (45, 54). Other cases where causal  
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1 association is likely to run in both directions or suitable instruments are unavailable, results may be less  
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3 clearly interpreted. Work implicating BMI in the aetiology of activity patterns in young participants  
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5 recently illustrated this point - whilst the impact of BMI on activity was marked and likely to be real, the  
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7 reciprocal relationship (which is likely to be present) was not possible to either exclude or describe  
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9 precisely (55).  
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14 One of the key parts of the MR process is the referral of evidence for causal relationships generated  
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16 through the use of genetic data back to the existing observational estimates. A good example of this and  
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18 the potential impact of MR analyses can be seen in that of C-reactive protein and the marked differences  
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20 in effect estimates that have been generated from observational and MR analyses with respect to effects  
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22 on cardiovascular health outcomes(56, 57, 58, 59, 60). In this case, it is the contrast between MR derived  
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24 and observational estimates which provides information given a lack of support for strong observational  
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26 effects. In contrast to this, one of the paradoxically dissatisfying observations from MR analyses of BMI as  
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28 a risk factor is the breadth of apparently causal associations (bar perhaps dental caries and depression and  
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30 foetal overnutrition(61, 62, 63, 64).  
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36 Blood pressure and cardiovascular health outcomes serve to illustrate this well with both within study  
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38 (Figure 4) and between studies(9, 43), however this is the case elsewhere. Bone health(50), cancer(52),  
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40 asthma(49), T2D(27, 44), osteoarthritis(65) have all shown some level of agreement in the associations  
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42 delivered by the best available observational studies and MR. Indeed a novel approach to the examination  
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44 of BMI as a causal risk factor(66) looking across a large number of possible intermediate phenotypes was  
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46 able to chart a broad spread of BMI related effects. This highlights one of the key problems in the analysis  
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48 of BMI in that, unlike others so far, there seems to be an underlying causal contribution to a large number  
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50 of health related outcomes. This therefore leaves questions as to what are the underlying pathways and  
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52 mechanisms responsible for these apparently causal relationships flagged by the broad exposure "BMI"  
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54 and also as to the validity of these MR tests.  
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### BMI effects: breaking down pathways

Despite the demonstration of the likely causal relationships between BMI and IHD, it is possible that weight itself is not the causal agent in disease, rather that there are a suite of intermediate phenotypes between BMI and outcome that deliver risk. Work not dissimilar to that originally published exploring the downstream impact of genetic variation at the *FTO* locus on cardiometabolic traits (42) used multiple intermediate phenotypes and also the health outcomes T2D and CHD (44) to try and unpick the pathways of BMI effect. In a relatively large collection of European participants (4,407 T2D, 6,073 CHD, and 3,813 stroke cases) the causal effect of a change in BMI of  $1\text{kg}/\text{m}^2$  on fasting glucose, fasting insulin, interleukin-6, systolic blood pressure, reduced HDL-C and low-density lipoprotein cholesterol (LDL-C) was estimated alongside the change in odds for disease outcome given the same exposure. This work was able to identify a host of intermediate risk factor associations and related this to the strongest health outcome effects (T2D), although additional power was needed to obtain precise estimates of BMI effect on CHD.

Coming from a similar methodological approach, MR can be employed in network analyses to identify causal risk factors in efforts to locate refined targets for therapeutic intervention (67). Any network of observational associations can be explored by the use genetic variants that independently predict the nodes of that network. A simple exposition of this network MR approach has been applied to break down the association between BMI and IHD (believed to be causal from previous MR analysis (46)) through the use of available intermediate risk factors measured in a population and novel instruments for each of them that have come from new GWAS studies. In an example of this, the Copenhagen General Population Study (N=71,407) and the Copenhagen City Heart Study (N=10,314) and a case-control study, the Copenhagen Ischemic Heart Disease Study (N=5,262), have been used to attempt to further dissect the BMI/IHD association using MR and causal mediation analyses (68) (Figure 5). This work suggests that it is likely that BMI driven elevations of non-fasting remnant cholesterol and LDL-C, elevated blood pressure and possibly elevated non-fasting glucose levels may contribute causally to risk of IHD.

### Intermediate phenotype and the “omics” revolution

1 Mendelian randomization studies have generally focused on a limited number of intermediate  
2 phenotypes, but recent applications of omic technologies into large scale population-based studies  
3 present new opportunities for identifying predictive biomarkers and causal links between established  
4 phenotypes and disease outcomes (69, 70, 71, 72, 73, 74). This has particular gravity for the types of  
5 network analysis being proposed above where, the combination of large-scale genetics data (and  
6 successful associations) are able to provide genetic proxy measures for an equally large collection of  
7 pathway specific intermediate risk factors. There is of course no guarantee that use of multi-omic  
8 phenotype data will avoid any of the problems encountered in observational epidemiology, but in  
9 combination with MR approaches, there is an opportunity to undertake network MR at scale. Omic  
10 technologies are now generating phenotypic data at a staggering rate and the use of these data in large  
11 scale population-based studies is presenting new opportunity for identifying novel predictive biomarkers  
12 and causal links between established phenotypes and disease outcomes (75, 76, 77, 78, 79, 80).

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30 As an example of this, it is known that metabolite profiles are useful in the prediction of cardiometabolic  
31 disease (81, 82), but that their role as modifiable targets for intervention or causal mediators of disease  
32 risk remains unclear. It is also known that many metabolites have substantial heritability and that it is  
33 possible to find robust associations between genetic genetic variation and the same metabolite features  
34 (83, 84). Together, it is then possible to examine the causal role of risk factors (in this case BMI) in the  
35 formation of metabolomics profiles(85) and then in a second stage (currently not applied to BMI  
36 systematically) to consider the causal role of those BMI driven metabolites in disease outcomes. This is a  
37 process termed two-step MR(69) and when applied across multiple collections with measurements of BMI,  
38 intermediate phenotypes (such as metabolites) and health outcomes, has the potential to informatively  
39 reduce the omics measure data space (to a set of anchoring genetic variants(39)) and to breakdown causal  
40 pathways to disease.

#### 51 52 53 54 55 56 **Challenges and limitations in the causal analysis of BMI**

57 *(i) Measurement – the idiosyncrasy of human phenotyping*  
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1 The options available to both clinicians and researchers for measuring adiposity are many and varied.  
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3 They range from simple indices of body weight for stature (e.g. BMI as focused on here) to detailed  
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5 imaging protocols using magnetic resonance imaging (MRI), computed tomography (CT), ultrasound, DXA  
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7 and electron microscopy. As well as permitting the differentiation of body fat compartments relevant to  
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9 health, such as subcutaneous versus visceral fat, such advanced technologies as combined positron  
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11 emission tomography (PET)-CT, allow even finer resolution such that quantities of brown fat (containing  
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13 mitochondria-rich brown adipocytes) can be measured.  
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19 The statistical construct that is BMI ( $\text{weight(kg)/height(m)}^2$ ) was first proposed as an index of relative  
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21 bodyweight by Adolphe Quetelet in 1842 (86). Promoted more formally in 1972, BMI was suggested to be  
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23 the optimum derivation for weight given stature based on the fact that, in a population of healthy men  
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25 aged 18 to 60 years this index had lowest correlation with height and the highest correlation with  
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27 measures of body fatness(87). Perhaps surprisingly, considering the technological advances of recent  
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29 years, a recent commentary on the evolution of BMI came to much the same conclusion as that of Keys  
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31 over forty years ago – that BMI is “*a robust, useful and surprisingly accurate measure of fatness in ‘healthy’*  
32  
33 *adults*” (88).  
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38 However, there remain two serious limitations of BMI as a measure of adiposity that are likely to hinder  
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40 causal analyses. Firstly, there is the apparent inability of BMI to adequately describe body composition  
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42 and related to the specific characteristics of different subgroups of the population. Comparisons of BMI  
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44 both with alternative indices of body weight such as waist circumference, and with MRI and DXA derived  
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46 measures of body composition have shown that BMI fails to discriminate well between major contributors  
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48 to body composition. For example, studies have shown that short and tall individuals and those from  
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50 different ethnicities have similar but not identical body compositions (89, 90). This type of limitation has a  
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52 bearing on the generalisability of BMI as a measure and has driven the development of both alternative  
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54 measures of adiposity, such as a body shape index (ABSI)(91) and modifications to BMI itself, for example,  
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56 by optimising the power term for height to minimise its influence (92).  
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4 Secondly, BMI is clearly not specific. Whilst the correlation of BMI with health outcomes is undeniable, the  
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6 biological interpretation of these relationships is complex. This problem was rather eloquently described  
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8 by Wells who stated "*Paradoxically, it seems that the various limitations of BMI as a specific index of*  
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10 *adiposity may also be its strengths as a composite index of cardio-metabolic risk*" (88). But this concept of  
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12 BMI as a "*composite index of risk*" is what makes its use in causal analyses so challenging. It is of course not  
13  
14 impossible to consider the utility of specific genetic variants or collections thereof to help dissect more  
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16 specific components of BMI, though this has not been systematically undertaken to date.  
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21 *(ii) Undertaking MR - don't trust your genetic proxies*  
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24 Statistical power, correlation between genetic variants (linkage disequilibrium (LD)), the non-specificity of  
25  
26 genetic effects (pleiotropy), developmental plasticity (or "canalization"(93)) and population stratification  
27  
28 have all been recognised as potential limitations to the MR approach(38). However, it has become possible  
29  
30 to assess and overcome issues of statistical power, LD and population stratification through the  
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32 combination of large data sets which are based in homogeneous population based collections and that use  
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34 independent genetic variants for analyses. Canalisation and pleiotropy remain potentially serious  
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36 limitations. The former of these has yet to really escape the bounds of theory and if present, may only act  
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38 to nullify genetic associations before they are found. On the other hand, pleiotropy and more generally the  
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40 blind use of biologically complex genetic variation (and potentially large collections of complex genetic  
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42 proxies from GWAS studies) remains one of the real challenges to these applied approaches.  
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47 It is becoming increasingly clear that there are important potential complications in the formation of  
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49 genetic proxy measures for MR studies. Through either the analysis of complex or derived phenotypic  
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51 outcomes which can generate genetic associations which are driven by artefactual biases(94) or just the  
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53 presence of complex biological underpinnings, the chosen genetic variation for MR studies may bring just  
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55 as many complications as they appear to avoid. In circumstances where well-characterised, candidate  
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57 driven and biologically understood genetic variants as proxy measures (relied upon in previous MR studies  
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1 derived from smaller scale genetic association studies) are unavailable, but where extremely large GWAS  
2 consortia yield apparently reliable association signals, it is tempting to use exhaustive lists of genetic risk  
3 factor in a genetic risk scores to undertake MR analyses (95).  
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10 Taking the example of educational attainment (a complex, biologically distal and poorly measured  
11 phenotype not dissimilar to BMI), a large-scale GWAS identified three genetic variants reliably correlated  
12 with education (96) but these signals represent less than a tenth of the expected difference between girls  
13 and boys in educational attainment (97). Faced with the lack of a strong genetic proxy for substantive MR  
14 study, an alternative strategy is to generate composite genetic proxy measures from collections of  
15 genomewide data (easily done through blind application of refined software interfaces such as PLINK  
16 (98)). In this example specifically, a composite genetic proxy measure for educational attainment can  
17 explain up to ~3% of the variance in this exposure would therefore be a valuable tool for MR studies  
18 focused on compelling hypotheses such as the impact of education on income/lifetime earning ability.  
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32 However, the formation of genetic proxy measures in this way can have complex flaws. Through the  
33 combined impact of genetic contributions from many different biological pathways and the possible  
34 biasing effects of pleiotropy, the use of genomewide proxy measures can produce effect estimates that  
35 are biased towards the confounded observational estimates MR is attempting to avoid. Furthermore, with  
36 the expansion of GWAS study sample size and power and the consequent discovery of increasingly distal  
37 contributions to outcome variance, looks set to introduce these complications even in the presence of  
38 apparently robust genetic association discoveries. The expansion of genetic association consortia for the  
39 analysis of BMI is now spilling way over  $n=300000$  and with targets of up to  $n=1000000$  in a single meta-  
40 analysis, the abundance of genetic proxy measures for BMI is set to grow. It is therefore with these  
41 limitations in mind that we should approach the use of novel findings that carry with them as much  
42 complication as clarification.  
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1 This is not to remove applied genetic epidemiology and MR as a logical extension to the analysis of causal  
2 relationships, rather to suggest that in an era of proliferation for genetic analysis, we should remain  
3 sceptical of the performance of any one analysis type. Triangulation of evidence should be sought where  
4 possible and MR viewed as a valuable contributor rather than a sole answer(37, 38). What is clear is that the  
5 success of MR and its move to mainstream analysis should not become the worst enemy of this approach.  
6 Furthermore, the growing presence of high quality functional biological data to help understand genetic  
7 associations and novel statistical approaches(99) to undertaking MR will help to relieve some of the  
8 problems mentioned above.  
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## 21 Conclusion

22 This review has considered major contributions to non-genetic approaches to assessing the causal impact  
23 of BMI on human health and current knowledge concerning the common, population based, genetic  
24 contribution to BMI and how this has fed into the developing field of applied epidemiology. We have  
25 revised complications of complex genetic aetiology and phenotypic measurement, and considered  
26 potential development and application of multiple omic data sources to help unpick the largely  
27 misunderstood relationships between BMI and human health and disease. Lastly, we have brought to  
28 attention the importance of appropriate use of applied genetic analyses in that whilst potentially complex,  
29 the ability to de-confound and add clarity to the prevailing weight of evidence is a superb possibility in  
30 suitable conditions.  
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45 Obesity and adiposity, measured principally via the faithful stand-in BMI, is of course a major risk factor  
46 when considering variance in risk for all sorts of health outcomes. There have now been a series of  
47 established study designs (prospective observational studies and MR analyses in particular) which have  
48 supported the notion of BMI as a causal agent in the formation of disease risk. For any given patient,  
49 however, it is unlikely to be the label "33kg/m<sup>2</sup>" that causes morbidity or mortality. Understanding the  
50 detailed routes from the biology reported (on average) by BMI to disease by employing new measurement  
51 techniques and through advanced causal analysis methods will be crucial for future preventative medicine.  
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2 Combinatorial investigations incorporating multi-omic examination of patients going through radical  
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4 changes in BMI via surgical intervention, population based analyses of BMI affect through MR and analyses  
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6 aimed at identifying modifiable risk factors able to modify exposure will be essential to the future  
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8 breakdown and understanding of how BMI exerts a causal effect on health.  
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51 **Figure titles:**

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53 **Figure 1:**

54 **Manhattan plot showing body mass index (BMI)-associated variants with loci identified prior to 2015**  
55 **in blue and novel loci identified by Locke *et al.* (33) in red.** Novel loci are labelled with the nearest gene,  
56 and the y-axis is truncated to allow easier observation of novel associations. This plot is reproduced from  
57 Locke *et al.* (33) with the permission of the authors.  
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**Figure 2:**

**The interplay between increased variance explained and diminishing marginal return as the number of confirmed body mass index (BMI)-associated genetic loci has increased.** The single line represents the cumulative variance explained and the double line the marginal return, calculated as the cumulative variance explained divided by the number of loci (29, 33, 100, 101, 102).

**Figure 3:**

**Mendelian randomisation; the use of genetic proxy measures of risk factors to allow causal inference.**

(A) In general, a genotype of use to this study is associated with the exposure, is independent of measured or unmeasured confounders and can only influence outcome via the causal effect of the exposure.

(B) The presence or absence of association between the BMI associated genotype and disease risk (from existing genomewide association study data sets) give evidence that the BMI is a causal risk factor for disease.

(C) Here genotype acts as a proxy measure for an exposure potentially affecting the BMI in a reciprocal analysis. This type of reciprocal analysis allows for a triangulation or network approach to the assessment of the effects of and effects on BMI.

**Figure 4:**

**The comparison of observational and Mendelian randomisation derived estimates for blood pressure and ischaemic heart disease.**

(A) Linear relationships between body mass index and blood pressure derived from observational and Mendelian randomization analyses. Upper scatter indicates systolic blood pressure and the lower diastolic. Grey areas around the estimated relationships indicate 95%CI for Mendelian randomisation estimates and in black those for observational estimates (plot generated from analysis for (43)). Note that for this analysis the log of body mass index was regressed on sex, age, age squared, log(height), and an age-sex interaction and exponentiated to give an individual's "relative BMI," that is, the ratio between his or her actual BMI and that expected for his or her sex, age, and height.

(B) Meta-analysis forest plots of observational and instrumental variable estimates of the relationship between ischaemic heart disease and body mass index. Odds ratios are for a 4kg/m<sup>2</sup> increase in body mass index (plot generated from analysis for (46)).

**Figure 5:**

**A two-step Mendelian randomization design applied to intermediate phenotype analysis in body mass index (BMI) and ischaemic heart disease (IHD).** In Step 1 (shown in red), BMI-associated variants are used to estimate the causal effect of BMI on relevant intermediates. In Step 2 (shown in green), variants associated with each of the intermediate traits are used to estimate the causal effect for those traits on IHD.

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FIGURE 1

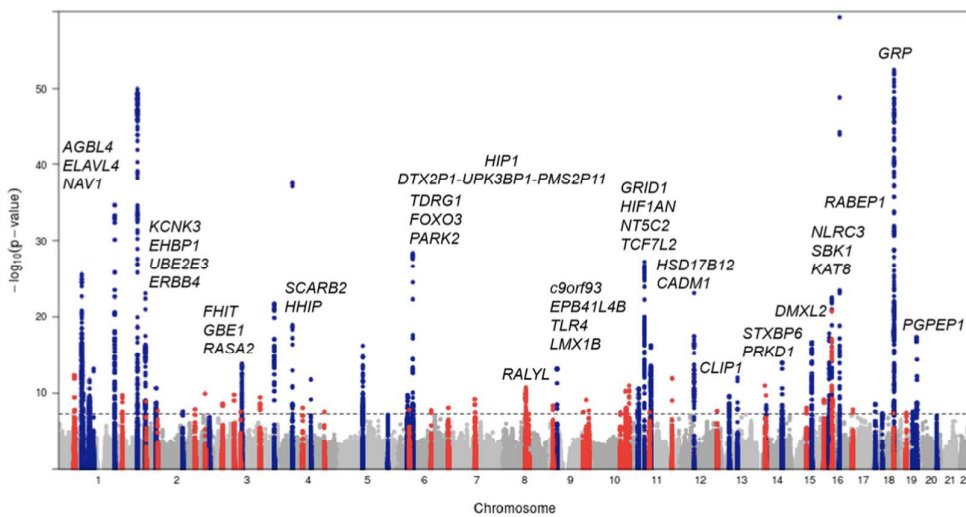
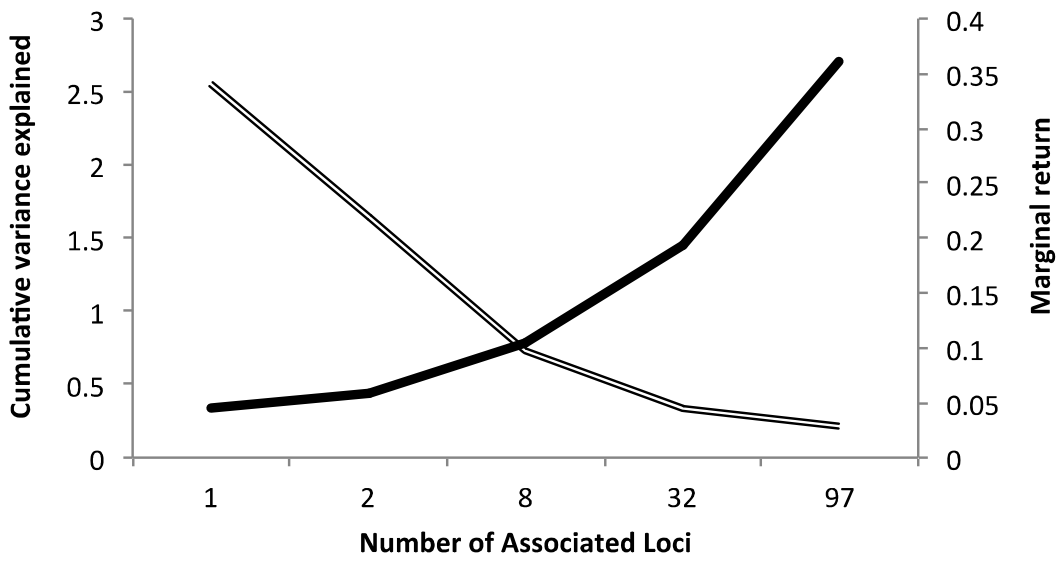
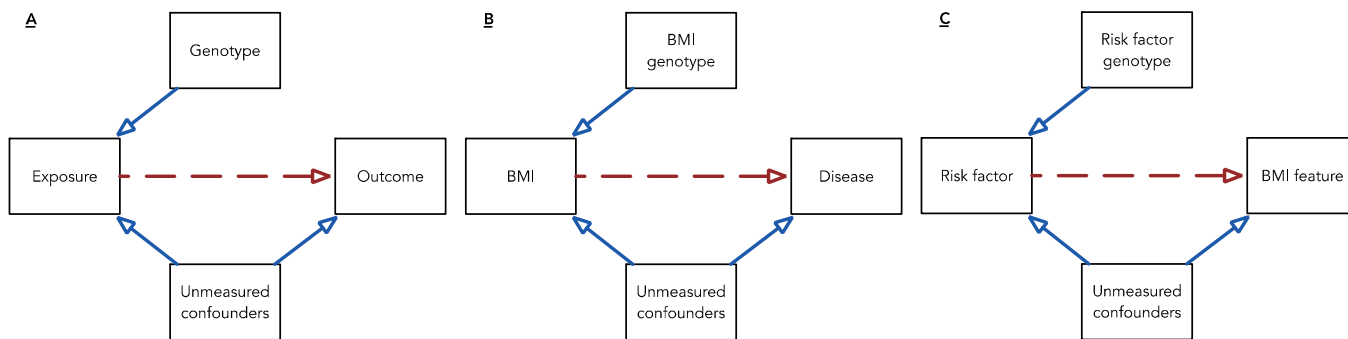


FIGURE 2



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FIGURE 3

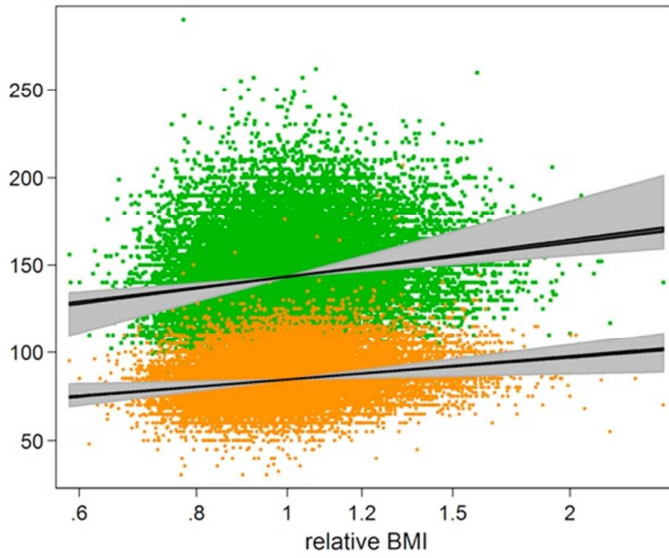


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FIGURE 4

**A**



**B**

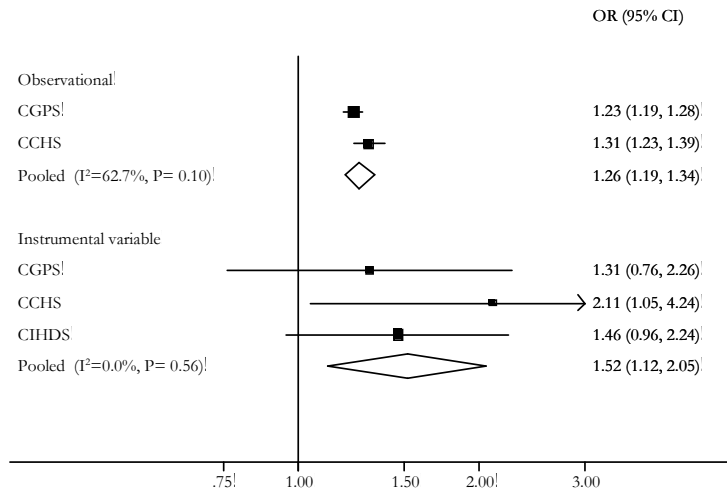
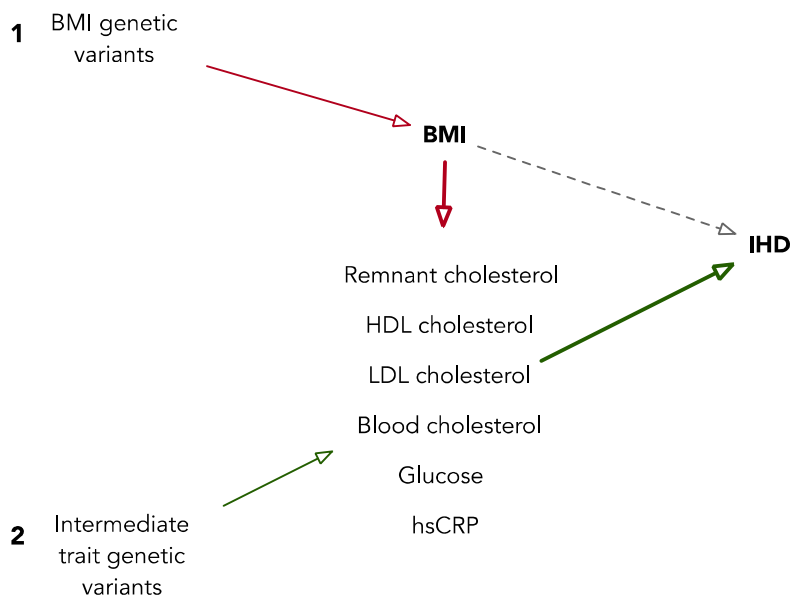


FIGURE 5



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