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Original Article

Mendelian Randomization Causal Analysis

# Adiposity as a cause of cardiovascular disease: a Mendelian randomization study

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## Abstract

**Background:** Adiposity, as indicated by body mass index (BMI), has been associated with risk of cardiovascular diseases in epidemiological studies. We aimed to investigate if these associations are causal, using Mendelian randomization (MR) methods.

**Methods:** The associations of BMI with cardiovascular outcomes [coronary heart disease (CHD), heart failure and ischaemic stroke], and associations of a genetic score (32 BMI single nucleotide polymorphisms) with BMI and cardiovascular outcomes were examined in up to 22 193 individuals with 3062 incident cardiovascular events from nine prospective follow-up studies within the ENGAGE consortium. We used random-effects

meta-analysis in an MR framework to provide causal estimates of the effect of adiposity on cardiovascular outcomes.

**Results:** There was a strong association between BMI and incident CHD (HR = 1.20 per SD-increase of BMI, 95% CI, 1.12–1.28, $P = 1.9 \cdot 10^{-7}$), heart failure (HR = 1.47, 95% CI, 1.35–1.60, $P = 9 \cdot 10^{-19}$) and ischaemic stroke (HR = 1.15, 95% CI, 1.06–1.24, P = 0.0008) in observational analyses. The genetic score was robustly associated with BMI ($\beta = 0.030$ SD-increase of BMI per additional allele, 95% CI, 0.028–0.033, $P = 3 \cdot 10^{-107}$). Analyses indicated a causal effect of adiposity on development of heart failure (HR = 1.93 per SD-increase of BMI, 95% CI, 1.12–3.30, P = 0.017) and ischaemic stroke (HR = 1.83, 95% CI, 1.05–3.20, P = 0.034). Additional cross-sectional analyses using both ENGAGE and CARDIoGRAMplusC4D data showed a causal effect of adiposity on CHD.

**Conclusions:** Using MR methods, we provide support for the hypothesis that adiposity causes CHD, heart failure and, previously not demonstrated, ischaemic stroke.

**Key words**: Cardiovascular disease, epidemiology, body mass index, Mendelian randomization

**Key Messages**

- We provide support for a causal association of adiposity with ischaemic stroke, which has not been observed in any previous studies.
- Earlier results on adiposity as a cause for heart failure are replicated and extended.
- We replicate and suggest a causal role of adiposity in coronary heart disease.

## Introduction

The increasing prevalence of obesity and overweight is a global problem,[1] and a number of epidemiological studies have established an association of adiposity, often measured as body mass index (BMI), with cardiovascular disease. Overweight ($BMI > 25\,kg/m^2$) and obesity were found to be associated with coronary heart disease (CHD), even after adjustments for traditional risk factors[2] although they should be seen as mediators rather than confounders.[3,4] The relationship between adiposity and stroke has not been as clear; however, a large combined analysis revealed an association of overweight with any stroke type,[5] which was later replicated for ischaemic stroke.[6] In addition, increased adiposity has been suggested to be an independent risk factor for the development of heart failure, in several large studies.[7,8]

Although observational studies have established correlations between adiposity and risk for cardiovascular disease, it is not yet clear whether adiposity has a causal role or is merely a surrogate marker for the true underlying factor. Moreover, results from previous interventional studies are inconclusive.[9,10] Negative results could be due to insufficient study sizes, follow-up time, or because the wrong indicator was used (general vs central adiposity), and highlights the complexity in the relationship.

The Mendelian randomization (MR) approach has the potential to investigate causal relationships between a risk factor and disease.[11] Observational studies often suffer from confounding, reverse causation (outcome influencing the exposure) or selection bias, all of which are difficult to control for and thus can lead to misinterpretation of results. Using genetic markers as instruments for a modifiable exposure, e.g. BMI, to make causal inference about a disease outcome has the potential to avoid these problems.[12] Recent studies that utilize the MR methodology have provided support for causal relations between increased adiposity and CHD[13] as well as heart failure,[14] although another MR study on the effect of adiposity on CHD found no such evidence.[15] However, the genetic instrument used in previous studies included a single or few genetic markers whereas, in contrast, a stronger genetic instrument based on a larger number of markers will yield better power and avoid weak-instrument bias.[16] In the present study, we utilized a genetic score derived from 32 established BMI-associated loci[17] as an instrument for lifelong BMI in order to more robustly investigate the causal association between BMI, here referred to as adiposity, and cardiovascular traits.[18] Towards this aim, we used a large prospective follow-up study to assess causality between adiposity and cardiovascular disease (CHD, heart failure and ischaemic stroke) using MR methods.

## Methods

### Study populations

The participating studies were recruited within the European Network for Genetic and Genomic Epidemiology (ENGAGE) consortium including 22 193 individuals with up to 3062 incident cardiovascular events from nine prospective studies (Tables S1, S2, available as Supplementary data at *IJE* online). Information on genotyping and quality control filters in each study is described in Supplementary data at *IJE* online. A non-weighted genetic risk score, as well as sensitivity analysis for a weighted score, was calculated from up to 32 independent BMI-associated single nucleotide polymorphisms (SNPs) reported by Speliotes et al.[17](Tables S3, S4, available as Supplementary data at *IJE* online).

### Outcomes

For each participant, the earliest available BMI measurement was used as baseline, and z-transformed for standardization, in each study. The cardiovascular outcomes were provided by the prospective follow-up studies and all were incident, i.e. occurring for the first time during follow-up (after baseline). The diagnoses were based on health registries and/or validated medical records (Table S5, available as Supplementary data at *IJE* online).

### Association analyses

Cox proportional hazards models were used to study associations of BMI and the genetic score with time from BMI measurement to incident cardiovascular disease. Linear regression models were fitted for the association of the genetic score with BMI (Section 4 of Supplementary Data at *IJE* online). The software used for statistical analysis within each cohort is listed in Table S2. To allow for heterogeneity between studies, random-effects models were used in the meta-analysis (Section 5 of Supplementary Data at *IJE* online).

### Instrumental variable analyses

The genetic risk score was used as the instrumental variable (IV) in the MR analysis, and the IV estimator was then calculated by dividing the corresponding untransformed beta from the meta-analysis of associations of genetic score with cardiovascular outcomes (separately for each outcome) by the beta from the meta-analysis of the association of the genetic score with BMI (Figure 1; Section 6 of Supplementary Data at *IJE* online).

### Secondary analyses

Secondary analyses were performed to study age at event and sex effects (Section 7 of Supplementary Data at *IJE* online). Each stratum was meta-analyzed separately before MR analyses were undertaken. To test for sex effects, the difference between the effect size estimates for men and women were calculated (Section 8 of Supplementary Data at *IJE* online).

Additional cross-sectional analyses in ENGAGE (Sections 4.2, 7.2 and 9 of Supplementary Data at *IJE* online) and

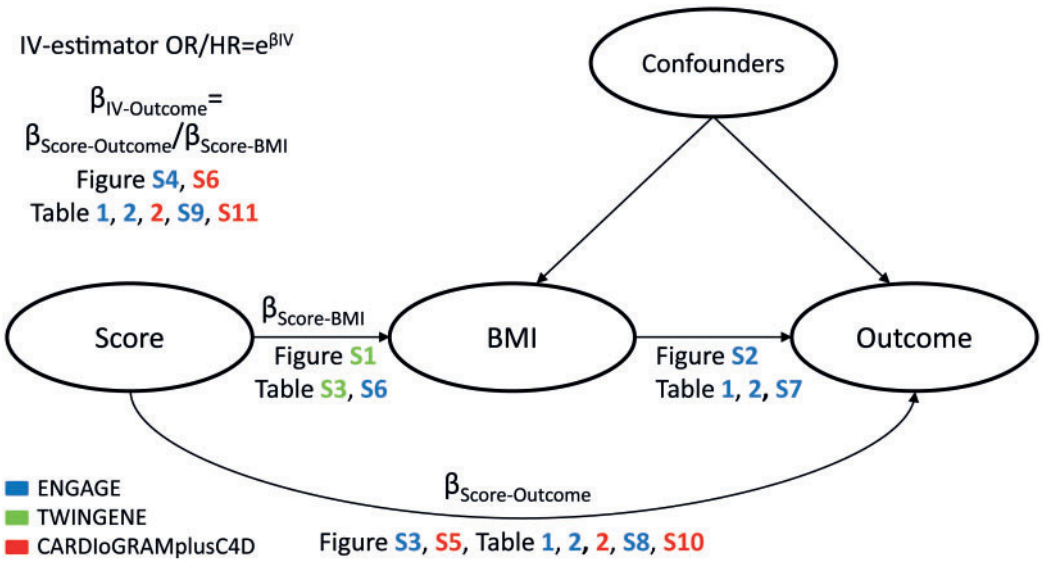


**Figure 1.** Directed acyclic graph explaining the relationships between exposure (BMI) and outcome (cardiovascular disease) with the genetic instrument (genetic score). The genetic risk score comprising up to 32 BMI-associated SNPs was associated with BMI and further with cardiovascular disease, and a non-confounded instrumental variable (IV) was calculated providing estimates for causal associations between BMI and outcome. Data used for the analyses were primarily ENGAGE cohorts, with sensitivity analyses in TWINGENE, and in addition CARDIoGRAMplusC4D consortium data.

CARDIoGRAMplusC4D data (Section 10 of Supplementary Data at *IJE* online), including sensitivity analysis for pleiotropic effects (Figure S7, available as Supplementary data at *IJE* online), are described in the Supplementary material. Here, cardiovascular outcomes were binary, so the relationships between BMI and outcomes as well as between genetic score and outcome were modelled via logistic regression.[19]

## Results

### Association analyses

The random-effects meta-analysis confirmed the association between the genetic score and BMI ($\beta = 0.030$ SD increase of BMI per allele, 95% CI, 0.028–0.033, $P = 2.77 \cdot 10^{-107}$; Table S6, available as Supplementary data at *IJE* online). The sample size weighted mean BMI was 25.9 kg/m$^2$ (SD 4.5) and the sample size weighted mean age was 49.5 years (SD 12.2) in all cohorts. The observational meta-analyses showed that higher BMI was associated with higher risk of incident CHD (HR = 1.20 per SD increase of BMI, 95% CI, 1.12–1.28, $P = 1.88 \cdot 10^{-7}$), heart failure (HR = 1.47 per SD increase of BMI, 95% CI, 1.35–1.60, $P = 9.27 \cdot 10^{-19}$) and ischaemic stroke (HR = 1.15 per SD increase of BMI, 95% CI, 1.06–1.24, $P = 0.00076$; Table 1; Figure S2, available as Supplementary data at *IJE* online). The genetic risk score meta-analysis for associations with outcome were for incident CHD (HR = 1.00 SD increase of BMI per allele, 95% CI, 0.99–1.02, $P = 0.62$), heart failure (HR = 1.02 SD increase of BMI per allele, 95% CI, 1.00–1.04, $P = 0.017$) and ischaemic stroke (HR = 1.02 SD increase of BMI per allele, 95% CI, 1.00–1.04, $P = 0.034$; Table 1; Figure S3, available as Supplementary data at *IJE* online).

### Instrumental variable analysis

The IV analyses suggested a causal effect of adiposity on incident heart failure (HR = 1.93, per SD increase of BMI, 95% CI, 1.12–1.30, $P = 0.017$) and ischaemic stroke (HR = 1.83 per SD increase of BMI, 95% CI, 1.05–3.20, $P = 0.034$; Table 1). There was no support for a comparable causal effect of BMI on incident CHD (HR = 1.13 per SD increase of BMI, 95% CI, 0.70–1.84, $P = 0.62$; Table 1), despite *post hoc* power calculations indicating that the current design had greater power for CHD than for heart failure or ischaemic stroke (84% power assuming HR = 2, similar to the estimated effects for the other outcomes; Section 11, Table S12, available as Supplementary data at *IJE* online). Despite the large differences in point estimates between observational and IV estimators, especially for ischaemic stroke (HR = 1.147 compared with 1.827), we could not provide statistical evidence because of overlapping confidence intervals (Table 1).

### Secondary analyses

We performed analyses stratified by age at event (cut-off 55 years) and sex to investigate differences between these groups. The IV analysis found strong associations of BMI with incident heart failure in women only (HR = 3.33 per SD increase of BMI, 95% CI, 1.60–6.93, $P = 0.001$) and with ischaemic stroke in men only (HR = 2.01 per SD increase of BMI, 95% CI, 1.02–3.98, $P = 0.04$) (Table S9, Figure S4, available as Supplementary data at *IJE* online). However, z-tests provided little support for a true sex difference in heart failure ($HR_{men\text{-}women} = 2.35$, 95% CI, 0.87–6.36, $P = 0.09$) or in ischaemic stroke ($HR_{men\text{-}women} = 1.95$, 95% CI, 0.47–8.13, $P = 0.36$). Overall, the results were driven by the late-onset disease events, as estimates in the early-onset strata were either unavailable due to insufficient number of events, or came with wide confidence intervals (Table S9; Figure S4).

Cross-sectional analyses in the ENGAGE data revealed consistent observational estimates of the BMI–cardiovascular association with the main analyses (Table S7). The IV estimate supported a causal association between BMI

**Table 1.** Meta-analysis results of Mendelian randomization analyses on effect of adiposity on cardiovascular disease

| CVD outcomes | Number of studies | Number of events[a] | Total numbers[a] | Observational results | | Genetic score–CVD | | IV Estimator | | Difference IV/BMI–CVD *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | HR (95% CI)[b] | *P*-value | HR (95% CI)[c] | *P*-value | HR (95% CI)[†] | *P*-value | |
| Coronary heart disease | 9 | 3062 | 22193 | 1.199 (1.120–1.284) | $1.88*10^{-7}$ | 1.004 (0.989–1.019) | 0.62 | 1.130 (0.695–1.837) | 0.62 | 0.81 |
| Heart failure | 7 | 1652 | 19384 | 1.473 (1.352–1.604) | $9.27*10^{-19}$ | 1.020 (1.004–1.037) | 0.017 | 1.925 (1.123–3.300) | 0.017 | 0.34 |
| Ischaemic stroke | 8 | 1500 | 20055 | 1.147 (1.059–1.243) | 0.00076 | 1.019 (1.001–1.036) | 0.034 | 1.827 (1.045–3.195) | 0.034 | 0.11 |

CVD, cardiovascular disease; BMI, body mass index; IV, instrumental variable; HR, hazard ratio; CI, confidence interval.
[a]Numbers from the association between genetic score and CVD.
[b]Increase per SD unit of BMI.
[c]SD increase of BMI per allele.

and prevalent CHD [odds ratio (OR) = 1.47, 95% CI, 1.04–2.07, $P = 0.03$] (Table 2; Table S9). In addition, we included data on CHD from the CARDIoGRAMplusC4D consortium[20] and performed equivalent analyses (Figure 1; Supplementary Data, Section 10, available at *IJE* online). The associations were strong for the genetic BMI effect on prevalent CHD (OR = 1.010 per BMI-increasing allele, 95% CI, 1.007–1.014, $P = 7.9{\cdot}10^{-9}$; Table 2; Figure S5, available as Supplementary data at *IJE* online) as well as for the IV effect on prevalent CHD (OR = 1.40 per SD increase of BMI, 95% CI, 1.24–1.58, $P = 2.4{\cdot}10^{-8}$; Table 2; Figure S6, available as Supplementary data at *IJE* online) with similar effect sizes as in ENGAGE. Moreover, IV estimators were also calculated for each SNP separately to illustrate possible effect dissimilarities (Figure S6).[18]

## Discussion

The present study utilized an MR design applied to studies within the ENGAGE consortium to address the causal role of adiposity in cardiovascular aetiology. Our main findings are several. First, we provide support for the first time that adiposity is causally related with ischaemic stroke. Second, we have replicated our earlier finding[14] of a causal role for adiposity in the development of heart failure. Third, using additional data from ENGAGE and CARDIoGRAMplusC4D, we suggest a causal association between adiposity and prevalent CHD.

### Comparison with other MR studies

There are a few previous studies that have addressed the causality of adiposity on cardiovascular disease. Recently, we published a paper using the rs9939609 *FTO* variant as an IV in MR analyses of cardiometabolic traits.[14] The study provided evidence for causality on heart failure, an observation that we now replicated using a genetic risk score providing more precise causal estimates and increased power. In the previous study, we did not find evidence for causal effects of BMI on ischaemic stroke, which we now could establish.

Another MR study by Nordestgaard and co-workers proposed a causal association of BMI on CHD using a genetic score derived from three SNPs.[13] We used a genetic score derived from 32 SNPs, and thus have a stronger instrument than Nordestgaard and co-workers; nevertheless, the effect estimates are similar to our cross-sectional results. However, they included a larger study sample with more CHD events collected during a long follow-up time, which resulted in a higher overall statistical power than our present study. Otherwise, the studies were comparable in terms of age, BMI and sex distribution.

In 2014, Holmes and co-workers presented an MR analysis of BMI on cardiometabolic traits.[15] They used a genetic score comprising 14 SNPs selected based on their genetic association study of BMI using the CardioChip.[21] They were unable to provide any support for a causal association between BMI and stroke, or between BMI and CHD.

Another way to address causality for adiposity-related outcomes is to include offspring BMI as an instrument of an individual's own BMI to avoid reverse causation. This has been illustrated by Davey Smith and co-workers, who concluded that estimates for associations using offspring BMI and cardiovascular mortality rates are higher than traditional observational estimates.[22]

### Adiposity and ischaemic stroke

Adiposity increases the risk of hypertension and type 2 diabetes, which in turn are risk factors for ischaemic stroke[6]. The underlying pathological processes of adiposity on ischaemic stroke could be atherosclerosis, disturbed blood flow and atherogenesis. Ischaemic stroke has been positively associated with adiposity in large observational studies;[5,6,23] thus, our causal estimate is in line with previous findings. The fact that we only estimate a reliable causal effect between adiposity and ischaemic stroke in men and not in women may partly be due to fewer events in women and lower power. Although we did find a fairly large effect difference between sexes, in line with previous findings,[24] the lack of precision precludes us from offering firm evidence.

**Table 2.** Cross-sectional associations between adiposity and coronary heart disease in ENGAGE and CARDIoGRAMplusC4D

| Coronary heart disease | ENGAGE | | CARDIoGRAMplusC4D | |
|---|---|---|---|---|
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Observed[a] | 1.241 (1.184–1.301) | $1.64{*}10^{-19}$ | – | – |
| Score[b] | 1.012 (1.001–1.022) | 0.03 | 1.010 (1.007–1.014) | $7.91{\cdot}10^{-9}$ |
| IV[a] | 1.466 (1.040–2.066) | 0.03 | 1.401 (1.245–1.577) | $2.38{\cdot}10^{-8}$ |

OR, odds ratio; CI, confidence interval; IV, instrumental variable.
[a]Increase per SD unit of BMI.
[b]SD increase of BMI per allele.

## Adiposity and heart failure

Adiposity has been shown to be a risk factor for heart failure,[7,8] and the association is likely to be mediated through increased blood pressure, dyslipidaemia or insulin resistance. Our previous study, using a different transformation of BMI which resulted in a different OR, indicated a causal relationship,[14] and the present study further strengthens the evidence for a causal association by using a genetic score as IV. Sex differences in the relation between adiposity and heart failure have been observed in previous epidemiological studies, which have described a somewhat higher risk in women using sex-specific baseline modelling.[25,26] This agrees with our estimates, even though the precision for the difference is again low. However, the fact that we detected a stronger effect in women, although our calculation showed more power in men, might lend some additional support for these earlier findings.

## Adiposity and coronary heart disease

The association between adiposity and CHD has been thoroughly studied in the past decades.[2,27]. The underlying cause of CHD is atherosclerosis, which provides a plausible mechanistic link for the relationship with adiposity. Randomized intervention trials of weight loss have been inconclusive.[9,10] However, it should be noted that atherosclerosis is driven by a long-term, low-grade inflammatory process, and short-term interventions on adiposity late in life might not reflect the same exposure as indicators of adiposity in MR studies. Further adding to the uncertainty, a meta-analysis of published MR studies could not provide evidence for a causal link between adiposity and CHD,[15] although the likely reason was underpowered analyses. In the present study, we suggest a causal association using cross-sectional ENGAGE and CARDIoGRAMplusC4D data. The effect size was smaller for CHD than for ischaemic stroke and heart failure, which likely explains the lack of significant effects in previous smaller MR studies.

## Strengths and limitations

Strengths of the present investigation include a large sample size with a large number of incident cardiovascular events, age- and sex-stratified analyses, high quality follow-up data and a strong IV based on multiple genetic variants.

However, there are also potential limitations to our investigation. Different disease definitions are used and some cohorts might have selection bias from genotyping at follow-up and not at baseline. Caution should always be taken regarding the assumptions of MR studies.[19,28] First, the genetic variants used as proxies for adiposity must have a reliable and independent association with BMI. Here, we report a strong association between genetic score and BMI, for variants robustly related to BMI.[17] Worth noting, however, is that there is a partial overlap in studies that contributed to this effort and to the discovery of the BMI-associated loci in the Speliotes *et al.* paper.[17] The SNPs are independent of confounders given the randomization during meiosis and conception, and analyses in TWINGENE showed no associations between the genetic score and smoking, education or exercise (Table S13, available as Supplementary data at *IJE* online). Population stratification is unlikely to be an issue because we include only individuals of European ancestry. Second, if the causal pathways from genotypes to cardiovascular outcomes do not go through adiposity, one of the assumptions would be violated. The well-known variants in the *FTO, MC4R* and *TMEM18* loci have been reported to have biological functions important for adiposity.[17,29,30] It is possible that many of the other BMI loci that are not yet well annotated will also be found to be of importance for biological mechanisms underlying adiposity, although at this point this is unknown. Third, no other phenotypes should be related to variants outside the causal pathway; i.e. there should be no pleiotropy. By investigating effects of individual adiposity SNPs on CHD using CARDIoGRAMplusC4D data, we could conclude that large pleiotropic effects were unlikely. We also conducted sensitivity analysis in CARDIoGRAMplusC4D excluding SNPs from the genetic score with tendency of outlying effect size in the IV estimator, with similar results (Figure S7, available as Supplementary data at IJE online). Fourth, there should be a log-linear association between the exposure and outcome which is not true for BMI in observational studies.[5,31] However, for a BMI value $\geq 25\,kg/m^2$, the association has been reported to be linear and therefore our findings are primarily applicable to those individuals. In any case, if linearity would infer bias, it would most likely lead to underestimation of the associations (as estimates would be driven towards the null). Fifth, observational studies could suffer from confounding at baseline where time from and age at BMI measurement could infer regression dilution bias. This type of error is avoided in the MR method where genetic variants are used as proxy for life-course BMI changes.[28]

## Conclusions

The use of MR methods to draw conclusions on non-confounded causal inference in large population-based studies is rapidly gaining ground. By use of a multiple variant genetic score instrument as a proxy for the intermediate phenotype, it is possible to enhance power in the analyses and to suggest causal effects in disease aetiology.

In the current study, we used data from individuals of European descent to provide support for a causal relationship between adiposity and CHD, heart failure and, for the first time, ischaemic stroke. Although we present the largest study of adiposity as a causal risk factor for ischaemic stroke so far, the confidence intervals were wide, and future larger studies are called for to further establish this relation.

## Supplementary Data

Supplementary data are available at *IJE* online.

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