



Mendelian Randomization Causal Analysis

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

Sara Hägg,^{1,2†} Tove Fall,^{1,2†} Alexander Ploner,¹ Reedik Mägi,³ Krista Fischer,³ Harmen HM Draisma,^{4,5} Mart Kals,^{3,6} Paul S de Vries,^{7,8} Abbas Dehghan,^{7,8} Sara M Willems,⁹ Antti-Pekka Sarin,^{10,11} Kati Kristiansson,¹¹ Marja-Liisa Nuotio,¹¹ Aki S Havulinna,¹¹ Renée FAG de Bruijn,^{7,12} M Arfan Ikram,^{7,12,13} Maris Kuningas,^{7,14} Bruno H Stricker,^{7,14} Oscar H Franco,^{7,8} Beben Benyamin,^{15,16} Christian Gieger,¹⁷ Alistair S Hall,¹⁸ Ville Huikari,¹⁹ Antti Jula,²⁰ Marjo-Riitta Järvelin,^{19,21,22,23} Marika Kaakinen,¹⁹ Jaakko Kaprio,^{10,11,24} Michael Kobl,¹⁷ Massimo Mangino,²⁵ Christopher P Nelson,^{26, 27} Aarno Palotie,^{10,11,28,29} Nilesh J Samani,^{26,27} Tim D Spector,²⁵ David P Strachan,³⁰ Martin D Tobin,³¹ John B Whitfield,¹⁶ André G Uitterlinden,^{7,8,32} Veikko Salomaa,¹¹ Ann-Christine Syvänen,³³ Kari Kuulasmaa,¹¹ Patrik K Magnusson,¹ Tõnu Esko,^{3,28,34,35} Albert Hofman,^{7,8} Eco JC de Geus,^{4,5} Lars Lind,³⁶ Vilmantas Giedraitis,³⁷ Markus Perola,^{3,10,11} Alun Evans,³⁸ Jean Ferrières,³⁹ Jarmo Virtamo,¹¹ Frank Kee,⁴⁰ David-Alexandre Tregouet,^{41,42,43} Dominique Arveiler,⁴⁴ Philippe Amouyel,⁴⁵ Francesco Gianfagna,^{46,47} Paolo Brambilla,⁴⁸ Samuli Ripatti,^{10,11,24,49} Cornelia M van Duijn,^{7,8,50} Andres Metspalu,^{3,51} Inga Prokopenko,^{49,52,53} Mark I McCarthy,^{49,52,54} Nancy L Pedersen¹ and Erik Ingelsson^{2,49*}; for the European Network for Genetic and Genomic Epidemiology (ENGAGE) consortium

¹Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden,

²Molecular Epidemiology and Science for Life Laboratory, Department of Medical Sciences, Uppsala University, Uppsala, Sweden, ³Estonian Genome Center, University of Tartu, Tartu, Estonia,

⁴Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands,

⁵EMGO Institute for Health and Care Research, Amsterdam, The Netherlands, ⁶Institute of

Mathematical Statistics, University of Tartu, Tartu, Estonia, ⁷Department of Epidemiology, Erasmus

Medical Center, Rotterdam, The Netherlands, ⁸Netherlands Consortium for Healthy Ageing,

Netherlands Genomics Initiative, Leiden, The Netherlands, ⁹Department of Genetic Epidemiology,

Erasmus Medical Center, Rotterdam, The Netherlands, ¹⁰Institute for Molecular Medicine Finland,

University of Helsinki, Helsinki, Finland, ¹¹National Institute for Health and Welfare, Helsinki, Finland,

¹²Department of Neurology, and ¹³Department of Radiology, Erasmus Medical Center, Rotterdam,

The Netherlands, ¹⁴Inspectorate for Health Care, The Hague, The Netherlands, ¹⁵Queensland

Brain Institute, University of Queensland, Brisbane, QLD, Australia, ¹⁶QIMR Berghofer Medical

Research Institute, Brisbane, QLD, Australia, ¹⁷Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany, ¹⁸Division of Epidemiology, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK, ¹⁹Center for Life Course and Systems Epidemiology, University of Oulu, Oulu, Finland, ²⁰Department of Chronic Disease Prevention, National Institute for Health and Welfare, Turku, Finland, ²¹Department of Children, Young People and Families, National Institute for Health and Welfare, Oulu, Finland, ²²MRC Health Protection Agency (HPE) Centre for Environment and Health, Imperial College London, London, UK, ²³Unit of Primary Care, Oulu University Hospital, Oulu, Finland, ²⁴Hjelt Institute, University of Helsinki, Helsinki, Finland, ²⁵Department of Twin Research and Genetic Epidemiology, King's College London, London, UK, ²⁶Department of Cardiovascular Sciences, University of Leicester, Leicester, UK, ²⁷National Institute for Health Research, Glenfield Hospital, Leicester, UK, ²⁸Broad Institute, Cambridge, MA, USA, ²⁹Haartman Institute, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland, ³⁰St George's, University of London, London, UK, ³¹Department of Health Sciences, University of Leicester, Leicester, UK, ³²Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands, ³³Molecular Medicine and Science for Life Laboratory, Uppsala University, Uppsala, Sweden, ³⁴Department of Genetics, Harvard Medical School, Boston, MA, USA, ³⁵Division of Endocrinology, Children's Hospital Boston, Boston, MA, USA, ³⁶Department of Medical Sciences, Uppsala University, Uppsala, Sweden, ³⁷Department of Public Health and Caring Sciences, Rudbeck Laboratory, Uppsala, Sweden, ³⁸Centre for Public Health, Queen's University of Belfast, Belfast, UK, ³⁹Department of Cardiology, Toulouse University School of Medicine, Rangueil Hospital, Toulouse, France, ⁴⁰UKCRC Centre of Excellence for Public Health Northern Ireland, Queen's University of Belfast, Belfast, UK, ⁴¹Team Genomics & Pathophysiology of Cardiovascular Diseases, Sorbonne Universités, UPMC Univ Paris 06, Paris, France, ⁴²Team Genomics & Pathophysiology of Cardiovascular Diseases, INSERM, UMR_S 1166, Paris, France, ⁴³ICAN Institute for Cardiometabolism and Nutrition, Paris, France, ⁴⁴Department of Epidemiology and Public Health, University Hospital of Strasbourg, Strasbourg, France, ⁴⁵Institut Pasteur de Lille, Université Lille Nord de France, Lille, France, ⁴⁶Research Centre for Epidemiology and Preventive Medicine (EPIMED), Department of Clinical and Experimental Medicine, University of Insubria at Varese, Italy, ⁴⁷Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Italy, ⁴⁸Department of Experimental Medicine, University of Milano-Bicocca, Monza, Italy, ⁴⁹Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, ⁵⁰Netherlands Genomics Initiative, Leiden University Medical Center, Leiden, The Netherlands, ⁵¹Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia, ⁵²Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK, ⁵³Department of Genomics of Common Disease, School of Public Health, Imperial College London, London, UK and ⁵⁴Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford, UK

*Corresponding author. Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Box 1115, SE-751 85 Uppsala, Sweden. E-mail: erik.ingelsson@medsci.uu.se

†These authors contributed equally to this work.

Accepted 5 May 2015

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/m²) and obesity were found to be associated with coronary heart disease (CHD), even after adjustments for traditional risk factors² 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 CHD¹³ 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 loci¹⁷ 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

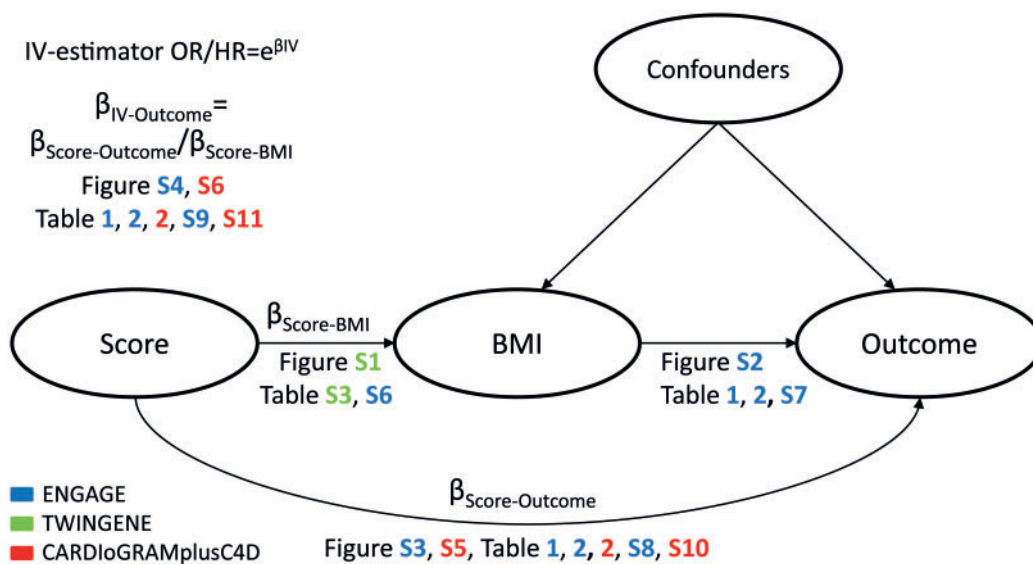


Figure 1. Directed acyclic graph explaining the relationships between exposure (BMI) and outcome (cardiovascular disease) with the genetic instrument (genetic score). The genetic risk score comprising up to 32 BMI-associated SNPs was associated with BMI and further with cardiovascular disease, and a 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/m² (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-women} = 2.35, 95% CI, 0.87–6.36, $P = 0.09$) or in ischaemic stroke (HR_{men-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.

^aNumbers from the association between genetic score and CVD.

^bIncrease per SD unit of BMI.

^cSD 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 consortium²⁰ 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 finding¹⁴ 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 stroke⁶. 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)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
Observed ^a	1.241 (1.184–1.301)	$1.64\cdot 10^{-19}$	–	–
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.

^aIncrease per SD unit of BMI.

^bSD 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 ≥ 25 kg/m², 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.

Acknowledgements

Acknowledgments and funding sources are listed in the Supplementary data.

Conflict of interest: None declared.

References

- World Health Organization. *Obesity and Overweight*. Fact sheet No. 311. Geneva: WHO, 2014.
- Bogers RP, Bemelmans WJ, Hoogenveen RT *et al*. Association of overweight with increased risk of coronary heart disease partly independent of blood pressure and cholesterol levels: a meta-analysis of 21 cohort studies including more than 300 000 persons. *Arch Intern Med* 2007;167:1720–28.
- Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet* 2014;23:R89–98.
- Varbo A, Benn M, Davey Smith G, Timpson NJ, Tybjaerg-Hansen A, Nordestgaard BG. Remnant cholesterol, low-density lipoprotein cholesterol, and blood pressure as mediators from obesity to ischemic heart disease. *Circ Res* 2015;116:665–73.
- Whitlock G, Lewington S, Sherliker P *et al*. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* 2009;373:1083–96.
- Strazzullo P, D'Elia L, Cairella G, Garbagnati F, Cappuccio FP, Scalfi L. Excess body weight and incidence of stroke: meta-analysis of prospective studies with 2 million participants. *Stroke* 2010;41:e418–26.
- Kenchaiah S, Evans JC, Levy D *et al*. Obesity and the risk of heart failure. *N Engl J Med* 2002;347:305–13.
- Kenchaiah S, Sesso HD, Gaziano JM. Body mass index and vigorous physical activity and the risk of heart failure among men. *Circulation* 2009;119:44–52.
- Wing RR, Bolin P, Brancati FL *et al*. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *N Engl J Med* 2013;369:145–54.
- Romeo S, Maglio C, Burza MA *et al*. Cardiovascular events after bariatric surgery in obese subjects with type 2 diabetes. *Diabetes Care* 2012;35:2613–17.
- Davey Smith G, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol* 2004;33:40–42.
- Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008;27:1133–63.
- Nordestgaard BG, Palmer TM, Benn M *et al*. The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a Mendelian randomisation approach. *PLoS Med* 2012;9:e1001212.
- Fall T, Hagg S, Magi R *et al*. The role of adiposity in cardiometabolic traits: a mendelian randomization analysis. *PLoS Med* 2013;10:e1001474.
- Holmes MV, Lange LA, Palmer T *et al*. Causal effects of body mass index on cardiometabolic traits and events: a mendelian randomization analysis. *Am J Hum Genet* 2014;94:198–208.
- Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int J Epidemiol* 2011;40:740–52.
- Speliotes EK, Willer CJ, Berndt SI *et al*. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* 2010;42:937–48.
- Palmer TM, Lawlor DA, Harbord RM *et al*. Using multiple genetic variants as instrumental variables for modifiable risk factors. *Stat Methods Med Res* 2012;21:223–42.
- Palmer TM, Sterne JA, Harbord RM *et al*. Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. *Am J Epidemiol* 2011;173:1392–403.
- Deloukas P, Kanoni S, Willenborg C *et al*. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* 2013;45:25–33.
- Guo Y, Lanktree MB, Taylor KC, Hakonarson H, Lange LA, Keating BJ. Gene-centric meta-analyses of 108,912 individuals confirm known body mass index loci and reveal three novel signals. *Hum Mol Genet* 2013;22:184–201.
- Davey Smith G, Sterne JA, Fraser A, Tynelius P, Lawlor DA, Rasmussen F. The association between BMI and mortality using offspring BMI as an indicator of own BMI: large intergenerational mortality study. *BMJ* 2009;339:b5043.
- Zhou BF. Effect of body mass index on all-cause mortality and incidence of cardiovascular diseases – report for meta-analysis of prospective studies open optimal cut-off points of body mass index in Chinese adults. *Biomed Environ Sci* 2002;15:245–52.
- Katsiki N, Ntaios G, Vemmos K. Stroke, obesity and gender: a review of the literature. *Maturitas* 2011;69:239–43.
- Ebong IA, Goff DC Jr, Rodriguez CJ *et al*. The relationship between measures of obesity and incident heart failure: the multi-ethnic study of atherosclerosis. *Obesity (Silver Spring)* 2013;21:1915–22.
- Loehr LR, Rosamond WD, Poole C *et al*. Association of multiple anthropometrics of overweight and obesity with incident heart failure: the Atherosclerosis Risk in Communities study. *Circ Heart Fail* 2009;2:18–24.
- National task force on the Prevention and Treatment of Obesity. Overweight, obesity, and health risk. *Arch Intern Med* 2000;160:898–904.
- Timpson NJ, Wade KH, Davey Smith G. Mendelian randomization: application to cardiovascular disease. *Curr Hypertens Rep* 2012;14:29–37.

29. Almen MS, Jacobsson JA, Shaik JH *et al.* The obesity gene, TMEM18, is of ancient origin, found in majority of neuronal cells in all major brain regions and associated with obesity in severely obese children. *BMC Med Genet* 2010; **11**:58.
30. Fredriksson R, Hagglund M, Olszewski PK *et al.* The obesity gene, FTO, is of ancient origin, up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. *Endocrinology* 2008;**149**:2062–71.
31. Chen Y, Copeland WK, Vedanthan R, Grant E, Lee JE, Gu D. Association between body mass index and cardiovascular disease mortality in east Asians and south Asians: pooled analysis of prospective data from the Asia Cohort Consortium. *BMJ* 2013;**347**: f5446.