Does Greater Adiposity Increase Blood Pressure and Hypertension Risk?
Mendelian Randomization Using the FTO/MC4R Genotype

Nicholas J. Timpson, Roger Harbord, George Davey Smith, Jeppe Zacho, Anne Tybjærg-Hansen, Børge G. Nordestgaard

Abstract—Elevated blood pressure increases the risk of experiencing cardiovascular events like myocardial infarction and stroke. Current observational data suggest that body mass index may have a causal role in the etiology of hypertension, but this may be influenced by confounding and reverse causation. Through the use of instrumental variable methods, we aim to estimate the strength of the unconfounded and unbiased association between body mass index/adiposity and blood pressure. We explore these issues in the Copenhagen General Population Study. We used instrumental variable methods to obtain estimates of the causal association between body mass index and blood pressure. This was performed using both rs9939609 (FTO) and rs17782313 (MC4R) genotypes as instruments for body mass index. Avoiding the epidemiological problems of confounding, bias, and reverse causation, we confirmed observational associations between body mass index and blood pressure. In analyses including those taking antihypertensive drugs, but for whom appropriate adjustment had been made, systolic blood pressure was seen to increase by 3.85 mm Hg (95% CI: 1.88 to 5.83 mm Hg) for each 10% increase in body mass index ($P$=0.0002), with diastolic blood pressure showing an increase of 1.79 mm Hg (95% CI: 0.68 to 2.90 mm Hg) for each 10% increase in body mass index ($P$=0.002). Observed associations are large and illustrate the considerable benefits in terms of reductions in blood pressure–related morbidity that could be achieved through a reduction in body mass index. (Hypertension. 2009;54:00-00.)

Key Words: epidemiology ■ genetics ■ blood pressure ■ obesity ■ fat mass

There is an approximately log-linear association between blood pressure (BP) and increased risk of cardiovascular events, and lowering BP in randomized, controlled trials yields a reduction in cardiovascular disease risk. Importantly, this reduction is not dependent on the nature of the antihypertensive therapy, strongly suggesting that it is BP lowering rather than other effects of therapeutic interventions that generates this benefit. This evidence strongly motivates public health approaches to reducing BP levels and the prevalence of hypertension within populations.

Obesity and higher body mass index (BMI) are known associates of BP and hypertension and related disease risks. If causal, elevated BMI is a key target for effective intervention with respect to the reduction of BP. Evidence as to the importance of obesity in relation to hypertension risk is available from trials of weight reduction and the effect of this on BP as a clinical outcome. Available meta-analyses of the relationship between weight loss and hypertension suggest that a reduction of weight by even relatively small levels can reduce the risk of hypertension reliably, but they do not comment on the possible causality in these relationships.

If causal, increases in BMI should lead to an increase in the burden of hypertension. However, increasing prevalence in obesity and average BMI level has been accompanied by secular decreases in BP level and prevalence of hypertension, reviews having questioned the nature of associations between obesity and hypertension. Thus, it has been pointed out that the randomized, controlled trials of weight reduction, which generally involve changes in dietary intake and/or exercise, could influence BP through mechanisms other than weight loss itself. Consequently, understanding the influence of obesity and elevated weight on BP and hypertension requires additional study, using methods that provide insights into the causal nature of the observed associations.

One approach to strengthening causal inference, that of mendelian randomization, is based on the proposition that association between a disease and a genetic polymorphism...
that proxies for a directly measured risk factor is not generally susceptible to the reverse causation or confounding. This technique is analogous to a randomized trial, in which randomization to genotype (and, thus, exposure) takes place at conception.\textsuperscript{15}

Recently, the application of this technique to questions regarding the role of obesity has been aided by the identification of genetic loci reliably associated with BMI/adiposity.\textsuperscript{16,17} After genomewide association studies for type 2 diabetes mellitus,\textsuperscript{16} the melanocortin 4 receptor locus (rs17782313,\textsuperscript{16}MC4R) and the melanocortin 4 receptor locus (rs17782313,\textsuperscript{16}MC4R). These provide suitable proxy markers for chronically elevated BMI and, importantly, markers that avoid both confounding and reverse causation. Together they account for $\sim$0.6% to 0.7% of observed variance in BMI in European populations, the greater part of the $\sim$1% now attributable to known, common, genetic polymorphisms.

The exact mechanisms of effect for these loci are not clear. The rs9939609\textit{FTO} locus may have a role in the hypothalamic regulation of appetite and food intake.\textsuperscript{18–20} However, the lack of complete understanding does not prevent the use of these independent loci to provide further evidence for the role of BMI in BP determination. Indeed, the\textit{FTO} locus has already been used in this way in the investigation of the role of greater weight in the determination of components of the metabolic syndrome.\textsuperscript{21}

Given the relationship between both\textit{FTO}/\textit{MC4R} and BMI, we aimed to assess the relationship between variation characterizing these associations and BP. Through the use of instrumental variable methods,\textsuperscript{22,23} we aim to estimate the strength of the unconfounded and unbiased association between BMI/adiposity and BP, together with the precision of such estimates.

### Subjects and Methods

#### Copenhagen General Population Study

This is a cross-sectional study of the Danish general population initiated in 2003 and still recruiting and with focus on multifactorial phenotypes, including BP. At the time of genotyping for the present study, 37,027 unrelated individuals had been included (response rate: 80%). All of the participants were white (Danish) and were selected based on the national Danish Civil Registration System to reflect the adult Copenhagen general population aged 20 to $\geq$80 years. The study was approved by Herlev Hospital and a Danish ethical committee, it adhered to the principles of the Declaration of Helsinki, and all of the subjects gave informed consent.

#### Genotyping

The ABI PRISM 7900HT Sequence Detection System was used to genotype the\textit{FTO} locus rs9939609 and the\textit{MC4R} locus rs17782313 using TaqMan assays. Genotyping was verified by DNA sequencing in $\geq$30 individuals with each genotype. We performed reruns twice, and 99.96% of all of the available participants were genotyped.

#### Outcome Variables

BMI was calculated as weight (kilograms) divided by height squared (meters squared). This was log transformed to reduce skewness. To remove the dependence of BMI on sex, age, and height, log (BMI) was regressed on sex, age, age squared, log(height), and an age-sex interaction. The residuals from this model give the difference between an individual’s actual log(BMI) and that expected for his or her sex, age, and height. Exponentiating residuals gives 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. BP was measured by trained technicians using an automatic Digital Blood Pressure Monitor (Kivex) on the left arm, after 5 minutes of rest, and with the subject in the sitting position. The inflatable part of the cuff was 22 $\times$32 cm; however, if the circumference of the upper arm was $\geq$46 cm, we used a 32 $\times$45-cm cuff.

Plots of the difference between cumulative frequency distributions by genotypes at both\textit{FTO} (rs9939609) and\textit{MC4R} (rs17782313) were used to examine the nature of relationships between these loci and relative BMI. These simultaneously assess both the effect of genotypes on BMI at all ranges of this outcome (an assumption important for the application of instrumental variable analyses\textsuperscript{22}) and also the most appropriate genetic model for the further use of genotypic data.

To adjust for the BP-lowering effect of antihypertensive medication, a constant value of 10 mm Hg was added to the systolic (diastolic) BP of those prescribed such medication.\textsuperscript{23} Any hypertension was defined as a systolic BP of $\geq$140 mm Hg, diastolic BP of $\geq$90 mm Hg, or the taking of antihypertensive drugs.\textsuperscript{24} Severe hypertension was taken as a systolic BP of $\geq$160 mm Hg, diastolic BP of $\geq$100 mm Hg, or the taking of antihypertensive drugs.

#### Other Covariates

Smoking and alcohol consumption were dichotomized and defined as “ever” (ex-smoker or current smoker) versus “never” smokers and drinkers as those consuming $\geq$36 g of alcohol per week. Other possible confounding factors included education (0 to 9 years, 10 to 12 years, or $\geq$13 years) and annual income ($\leq$400 000 krone, 400 000 to 600 000 krone, or $\geq$600 000 krone ($100 000 krone is approximately $15 000)).

#### Analyses

We used Stata 10 (Stata Corp), $P=0$ is equivalent to $P=10^{-200}$ or less. In observational and genetic analyses, continuous effects were estimated using linear regression with adjustment for age (quadratic), sex, height (logged), and an age-sex interaction (linear in age). Genotypes were used categorically. Logistic regression tested for association of the binary variable hypertension with tertiles of BMI and with\textit{FTO}/\textit{MC4R} genotypes, adjusted for age and sex.

For mendelian randomization analyses, instrumental variable methods were used to obtain estimates of the association between BMI and BP.\textsuperscript{27,28} This was performed using both rs9939609 (\textit{FTO}) and rs17782313 (\textit{MC4R}) as instruments for BMI and adjusting for age, sex, and height, as before. We used the generalized method of moments with robust standard errors to fit the instrumental variable models in the main analyses but checked results using limited-information maximum likelihood and 2-stage least squares. We compared the instrumental variable estimates with those from ordinary linear regression using the Durbin form of the Durbin-Wu-Hausman statistic. We examined F statistics from the first-stage regressions to evaluate the strength of the instruments.\textsuperscript{29–31}

#### Results

### Observational

Table 1 shows baseline characteristics. Observationally, there was strong evidence for a linear association between BMI and systolic/diastolic BPs. Waist:hip ratio was strongly correlated with BMI (correlation coefficient: 0.5; $P<0.001$), and observational results reflected this. The correlation coefficient between BP and log-relative BMI was 0.20 ($P<0.001$) and 0.24 ($<0.001$) for systolic and diastolic BPs, respectively (Figure S1, available in the online data supplement at http://hyper.ahajournals.org). When arranged into
deciles, the proportion of individuals found to have hypertension was also seen to increase consistently with BMI (Figure 1). Each tertile increase of BMI showed an accompanying increase in the odds of hypertension of 1.73 (95% CI: 1.68 to 1.78; P=0). This relationship was only slightly attenuated by adjustment for age, sex, education, smoking, and drinking (odds ratio: 1.71; 95% CI: 1.65 to 1.77; P=0). For a more strict definition of severe hypertension, each tertile increase of BMI led to an odds ratio of 1.72 (95% CI: 1.67 to 1.77; P=0). This was, again, slightly attenuated in the adjusted model (OR=1.68; 95% CI: 1.63 to 1.74; P=0).

**Genetics**

*FTO* rs9939609 was observed with a minor allele (A/fwd) frequency of 0.40 (SE: 0.002; counts: 13 019/18 057/5951). There was nominal evidence from a departure from Hardy-Weinberg equilibrium (P=0.02). *MC4R* rs17782313 was observed with a minor allele (C/fwd) frequency of 0.25 (SE: 0.002; counts: 21 011/13 717/2299). This variant was observed to adhere to Hardy-Weinberg equilibrium (P=0.3). In contrast to the observed relationship between possibly confounding factors and BMI, there were no robust associations between potentially confounding factors and Rs9939609 and rs17782313 genotypes in this cohort (Table S1).

Plots of the difference between the cumulative distribution functions of relative BMI stratified by both *FTO* and *MC4R* genotypes showed that both loci appeared to exert effects across the distribution of BMI (Figure 2). However, whereas *FTO* rs9939609 demonstrated differences in relative BMI cumulative frequency distribution (by genotype) that were indicative of an additive effect, *MC4R* rs17782313 showed no substantial difference in the level of difference between groups defined as major homozygote versus heterozygote and major homozygote versus minor homozygote (Figure 2). This suggested that an assumption of additivity may not be appropriate in the case of *MC4R* rs17782313 and that categorical analyses would be more appropriate for further analyses of both genotypes in this instance.

BMI showed an expected relationship with *FTO* rs9939609, with each rare allele accounting for a 1.18 (95% CI: 0.96 to 1.41) age- and sex-adjusted symmetrical percentage increase (P=2.0e-24; Table 2). The corresponding effect for the *MC4R* rs17782313 locus was a symmetrical percentage difference of 0.78 (95% CI: 0.53 to 1.04; P=2.2e-09) for BMI. For systolic BP, adjusted age and sex showed an increase of 0.63 mm Hg (95% CI: 0.33 to 0.93 mm Hg) per rare allele.

**Table 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th>Variable (n)</th>
<th>Median (SD) or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n=17 217/37 027)</td>
<td>46.5</td>
</tr>
<tr>
<td>Age, y (N=37 027)</td>
<td>57.60 (13.49)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg (N=37 011)</td>
<td>143.30 (22.42)</td>
</tr>
<tr>
<td>Diastolic BP (N=37 010), mm Hg</td>
<td>84.50 (11.91)</td>
</tr>
<tr>
<td>Hypertension (n/N=24 813/37 027)</td>
<td>67.0</td>
</tr>
<tr>
<td>Severe hypertension (n/N=11 656/37 027)</td>
<td>31.5</td>
</tr>
<tr>
<td>BMI, kg/m² (N=36 867)</td>
<td>26.20 (4.27)</td>
</tr>
<tr>
<td>Waist:hip ratio (N=37 027)</td>
<td>0.880 (0.089)</td>
</tr>
<tr>
<td>Drinkers (n/N=21 117/37 027)</td>
<td>57.0</td>
</tr>
<tr>
<td>Smokers (n/N=18 005/37 027)</td>
<td>48.6</td>
</tr>
<tr>
<td>Education (n=11 180, 14 329, 3623)</td>
<td>38.4</td>
</tr>
<tr>
<td>Income (n=14 445, 11 253, 3140)</td>
<td>50.1</td>
</tr>
</tbody>
</table>

Waist:hip ratio refers to a ratio of waist and hip measurements, both taken in centimeters.

*BP* was adjusted for antihypertensive medication by adding 10 mm Hg (systolic) and 5 mm Hg (diastolic).

†Proportion in the lowest group is shown, and numbers are shown for low-to-high educational and income level, respectively.

**Figure 1.** Relationship between the proportion of individuals with hypertension and BMI within the Copenhagen General Population Study cohort. Error bars represent 95% CIs for percentages.

**Figure 2.** Difference between cumulative distribution functions by genotype of *FTO* and of *MC4R* overlaid on the distribution of relative BMI. Solid line indicates the difference between cumulative distribution functions for heterozygotes and homozygotes for the major allele. Dashed line indicates the difference between cumulative distribution functions for homozygotes for the minor allele and homozygotes for the major allele. Density function (gray) estimated by kernel density estimation and differences smoothed using kernel-weighted local linear regression, both using Epanechnikov kernels of width 0.02 (on the log scale). Relative BMI is the ratio of actual BMI to predicted BMI for that person’s age, sex, and height from a linear regression model.
Instrumental variable analysis. The Durbin form of the Durbin-Wu-Hausman test was used to test the difference between the estimates from linear regression (without additional adjustment) and instrumental variable estimates. Moderate elevated BP is defined as systolic BP >100 mm Hg, diastolic BP >60 mm Hg, or being on antihypertensive medication. Severe elevated BP is defined as systolic BP >160 mm Hg, diastolic BP >100 mm Hg, or being on antihypertensive medication.

of FTOrs9939609 (P = 0.00004; Table 2). With this, diastolic BP showed an increase of 0.26 mm Hg (95% CI: 0.09 to 0.43 mm Hg) per rare allele at FTOrs9939609 (P = 0.003). For any and severe hypertension, the odds ratios per FTOrs9939609 allele were found to be 1.07 (95% CI: 1.04 to 1.11; P = 0.00007), respectively, in the same sex- and age-adjusted data set (Table 2).

For MC4Rrs17782313, BP did not show differences by genotype; however, it showed the direction of effect consistent with the expected relationships between MC4R and BMI. Age and sex adjustments showed an effect for systolic BP of 0.20 mm Hg (95% CI: −0.14 to 0.54 mm Hg) per rare allele at MC4Rrs17782313 (P = 0.3; Table 2). Diastolic BP showed an effect of 0.08 mm Hg (95% CI: −0.12 to 0.27 mm Hg) per rare allele at MC4Rrs17782313 (P = 0.4). For any and severe hypertension, the odds ratios per MC4Rrs17782313 allele were found to be 1.02 (95% CI: 0.99 to 1.06; P = 0.2) and 1.00 (95% CI: 0.96 to 1.04; P = 0.96), respectively, in the same sex- and age-adjusted data set (Table 2).

Relationships among FTOrs9939609, BP, and hypertension were maintained after adjustment for education, income, smoking, and drinking (Table 2). In contrast to this, when the association between FTOrs9939609 and BP was adjusted for BMI, relationships were considerably attenuated, and no convincing evidence for association by genotype was found. Similar patterns were seen in the case of MC4Rrs17782313 (Table 2).

**Mendelian Randomization**

There were strong relationships between tertiles of relative BMI and BP within this cohort (Table 3). In a linear regression model, systolic BP increased by 2.75 mm Hg (95% CI: 2.62 to 2.88 mm Hg) and diastolic BP by 1.75 mm Hg (95% CI: 1.68 to 1.83 mm Hg) for each 10% increase in BMI. Adjustment of these relationships for the confounding factors education, income, drinking, and smoking led to

**Table 3. Relationships of BMI With Systolic and Diastolic BPs as Tested by Both Conventional Epidemiological Approaches and Through the Application of Instrumental Variables Analysis**

<table>
<thead>
<tr>
<th>Variable (N)</th>
<th>Measured (95% CI)</th>
<th>Change per 10% Increase in BMI</th>
<th>Instrumental Variables (Adjusted for Sociobehavior), N*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic BP, mm Hg (N = 36 851)</strong></td>
<td>138.3 (137.9 to 138.7)</td>
<td>2.75 (2.62 to 2.88)</td>
<td>3.85 (1.88 to 5.83), 0.0002</td>
</tr>
<tr>
<td><strong>Diastolic BP, mm Hg (N = 36 851)</strong></td>
<td>81.3 (81.1 to 81.5)</td>
<td>1.75 (1.68 to 1.83)</td>
<td>1.79 (0.68 to 2.90), 0.002</td>
</tr>
</tbody>
</table>

BP measures incorporate adjustment for those on medication (addition of 10 mm Hg to systolic BP and 5 mm Hg to diastolic BP). Means (95% CI) and regression results were adjusted for age, sex, and height. Instrumental variable estimates use FTO and MC4R genotypes as instruments for BMI. P (DWH) is the P value for a test (the Durbin-Wu-Hausman test) for the difference between the estimates from linear regression (without additional adjustment) and instrumental variable analysis.

*Sociobehavioral variables were adjusted for income, education, smoking, and drinking (in addition to adjustment for age, sex, and height).
attenuation of associations (Table 3). This can be considered in light of the associations of BMI and potential confounding factors presented in Table S1. Both education and drinking behavior showed strong patterns of association with BMI tertile, with those in higher BMI groups being less likely to be in the highest drinking group but more likely to be in the lowest educational bracket.

In instrumental variable analyses for the assessment of the continuous risk factor BMI on BP, using both FTO rs9939609 and MC4R rs17782313 as instruments for BMI confirmed observational associations between BMI and BP. In analyses, including those of individuals taking antihypertensive drugs but for whom appropriate adjustment had been made, systolic BP was predicted to increase by 3.85 mm Hg (95% CI: 1.88 to 5.83 mm Hg) for each 10% increase in BMI (P = 0.0002; Table 3). In the equivalent analysis, diastolic BP showed an estimated increase of 1.79 mm Hg (95% CI: 0.68 to 2.90 mm Hg) for each 10% increase in BMI (P = 0.002).

For all of these analyses, the first-stage F statistic was >60,29,30 and there was no evidence of a departure of instrumental variable–derived estimates from those derived from observational analyses. Furthermore, effects from instrumental variable analysis were seen to have point estimates consistently greater than those derived from either basic or adjusted observational analyses. The correspondence between estimates derived from both observational and instrumental variable analyses is also shown in Figure 3.

In analyses using FTO rs9939609 and MC4R rs17782313 as instruments for BMI separately, statistically equivalent estimates for the association between a 10% elevation in BMI and BP were attained. The SEs derived from these separate estimates were considerably larger (SEs: 1.23 and 1.77, respectively) than that derived from the use of both of these instruments simultaneously (SE: 1.01).

Observational estimates of the relationship between BMI and BP declined markedly with age. In data not shown, the effect of BP was seen to approximately halve in those >75 years old compared to those younger (P = 0.007 interaction). In contrast to this, there was no evidence of an age-related change in the instrumental variable–derived BP/BMI effect. Instrumental variable analyses performed with the incorporation of drinking, smoking, education, age, and income as covariates in the instrumental variable model did not substantially alter results, nor did stratification by sex.

Discussion

As expected, strong observational associations were found between BMI and hypertension. A 10% elevation in BMI was seen to be associated with a 3-mm Hg increase in systolic...

Table 2. Continued

<table>
<thead>
<tr>
<th>Per-Allele Effects</th>
<th>Adjusted for Age and Sex Alone</th>
<th>P</th>
<th>Adjusted for Age, Sex, and Sociobehavior†</th>
<th>P</th>
<th>Adjusted for Age, Sex, and Log (BMI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.18 s% (0.96 to 1.41)</td>
<td>2.0e-24</td>
<td></td>
<td>1.11 s% (0.85 to 1.36) (N = 28 616)</td>
<td>1.3e-17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.63 (0.33 to 0.93)</td>
<td>0.00004</td>
<td></td>
<td>0.63 (0.29 to 0.97) (N = 28 718)</td>
<td>0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.26 (0.09 to 0.43)</td>
<td>0.003</td>
<td></td>
<td>0.34 (0.14 to 0.53) (N = 28 717)</td>
<td>0.0007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.07 (1.03 to 1.10)</td>
<td>0.0002</td>
<td></td>
<td>1.06 (1.02 to 1.10) (N = 28 717)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.07 (1.04 to 1.11)</td>
<td>0.00007</td>
<td></td>
<td>1.07 (1.03 to 1.11) (N = 28 717)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.78 s% (0.53 to 1.04)</td>
<td>2.2e-09</td>
<td></td>
<td>0.79 s% (0.50 to 1.08) (N = 28 616)</td>
<td>8.0e-08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20 (–0.14 to 0.54)</td>
<td>0.3</td>
<td></td>
<td>0.04 (–0.34 to 0.42) (N = 28 718)</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.08 (–0.12 to 0.27)</td>
<td>0.4</td>
<td></td>
<td>0.02 (–0.20 to 0.24) (N = 28 717)</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.02 (0.99 to 1.06)</td>
<td>0.2</td>
<td></td>
<td>1.01 (0.97 to 1.06) (N = 28 717)</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00 (0.96 to 1.04)</td>
<td>0.96</td>
<td></td>
<td>0.98 (0.94 to 1.02) (N = 28 717)</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Linear relationships between BMI and BP derived from both observational analysis and instrumental variable approaches. Upper scatter indicates systolic BP, whereas lower scatter indicates diastolic BP. Gray areas represents 95% pointwise confidence regions around instrumental variables estimates. Black area represents 95% pointwise confidence regions around simple linear regression estimates.
BP, this not only replicating previous findings but also adding to the weight of evidence as to the possibility of BP regulation through BMI control. However, we also found that BMI was robustly associated with socioeconomic factors, including educational status and alcohol consumption. As such, especially in light of alcohol consumption, which has been shown to be reproducibly associated with BP, the existence of confounding could not be ruled out in the explanation of these initial observations.

Within this population-based sample, the known relationships between BMI and the FTO and MC4R loci were replicated. Furthermore, these genotypes demonstrated 2 key properties that were important and informative to the formal undertaking of mendelian randomization analysis. First, both FTO and MC4R showed no consistent relationship with factors potentially confounding the BMI/BP relationship. Second, although the effect size of MC4R variation on BMI precluded a robust estimation of the direct genetic effect on BP, both genotypes showed directions of direct association with BP consistent with elevated BMI being positively and causally related to hypertension.

Previous work has noted that quantifiable increases in weight can be equated to expected increases in BP. Specifically, Neter et al. showed, from meta-analysis of weight reduction–intervention studies, that a reduction in weight of \( \approx 5 \) kg (by means of energy restriction, exercise, or both) was enough to achieve a reduction in systolic BP of \( \approx 4 \) mm Hg. Variation at FTO rs9939609 has been shown to be related to an \( \approx 2 \) kg difference in weight (from rare to common homozygote). Simple analysis shows an effect of just \( >1.0 \) mm Hg per rare FTO allele (systolic BP) in the Copenhagen cohort. This is roughly equivalent to a just \( >2.5 \) mm Hg increase in systolic BP per 5 kg of weight, and, although lower than previous estimates, it remains positive.

The application of instrumental variable analysis examining the relationship between BMI and BP gave an opportunity to appraise this association without the limitation of confounding, reverse causation, or measurement error. In this case, observational associations were supported by those associations found in a mendelian randomization framework. Furthermore and as expected from a lifelong exposure to elevated adiposity/BMI, there was actually evidence for greater estimates for the effects of BMI increase on BP increase when compared with those estimates derived from “one-off” cross-sectional analyses. This consistency in findings presents evidence that favors the consideration of BMI/adiposity increase as a causal factor in the etiology of hypertension. Also, unlike the naïve observational associations, there was no attenuation of the instrumental variable estimates with age.

Possible limitations to the undertaking of mendelian randomization here include the possibility of population stratification, canalization, power deficiency, and an inability to detect the effect of acute changes in BMI on BP. However, because of the nature of this population cohort, both population stratification and power are unlikely to have considerable impact. Although the effect of acute BMI changes on BP are not the major focus here, the possible effects of canalization are lessened because of both the apparent lack of effect of the FTO locus on adiposity in early life and the concordance of MC4R-derived results with those of FTO as an instrument for adiposity.

A further possible limitation of the approach taken in this work relates to the possible existence of pleiotropy concerning the genetic variation used as a proxy measure for BMI. Recent work concentrating on rare variation at the MC4R locus has suggested that there may be a BMI and insulin-independent effect on BP, and, as such, it may have the potential to introduce error to the inference taken from instrumental variable analysis. However, analyses here suggest an attenuation of the expected association between variation of the MC4R locus and BP (Table 2), suggesting that MC4R variation retains a BMI-specific BP effect (as seen for FTO).

### Perspectives

Overall, this research does not present a new hypothesis but adds to the available weight of evidence supporting a causal role for raised BMI in the etiology of hypertension. The exact mechanisms of this pathway may not be entirely clear; however, both observational and now mendelian randomization–derived data support the notion of targeting BMI directly as part of an effective therapeutic regime against hypertension.

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### Disclosures

None.

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DOES GREATER ADIPOSITY INCREASE BLOOD PRESSURE AND HYPERTENSION RISK? MENDELIAN RANDOMIZATION USING *FTO/MC4R* GENOTYPE.

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Table S1
Relationships between tertile of body mass index and *FTO/MC4R* genotype and possible confounding factors.

<table>
<thead>
<tr>
<th>Variable (n)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index tertile</td>
<td></td>
<td></td>
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<tr>
<td>Smoking (17918/36867)</td>
<td>46.30 (45.42, 47.18)</td>
<td>48.56 (47.67, 49.44)</td>
<td>50.95 (50.06, 51.83)</td>
<td>1.01 (0.99, 1.04)</td>
<td>0.3</td>
</tr>
<tr>
<td>Drinking (21052/36867)</td>
<td>56.33 (55.45, 57.2)</td>
<td>59.01 (58.14, 59.88)</td>
<td>55.97 (55.09, 56.84)</td>
<td>0.87 (0.85, 0.89)</td>
<td>6.4e-25</td>
</tr>
<tr>
<td>Education* (11126, 14273, 3614)</td>
<td>29.49 (28.58, 30.42)</td>
<td>37.31 (36.35, 38.28)</td>
<td>47.72 (46.74, 48.7)</td>
<td>0.70 (0.67, 0.72)</td>
<td>9.7e-106</td>
</tr>
<tr>
<td>Income* (4415, 4567, 5384)</td>
<td>47.57 (46.55, 48.58)</td>
<td>47.65 (46.65, 48.65)</td>
<td>54.62 (53.64, 55.6)</td>
<td>0.89 (0.86, 0.92)</td>
<td>2.5e-12</td>
</tr>
</tbody>
</table>

*FTO* genotypes (major homozygote/heterozygote/minor homozygote)

<table>
<thead>
<tr>
<th>Variable (n)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking (18005/37027))</td>
<td>48.29 (47.43, 49.15)</td>
<td>48.76 (48.03, 49.49)</td>
<td>48.97 (47.7, 50.24)</td>
<td>1.02 (0.99, 1.05)</td>
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<tr>
<td>Drinking (21117/37027)</td>
<td>57.6 (56.75, 58.45)</td>
<td>56.66 (55.94, 57.38)</td>
<td>56.91 (55.65, 58.17)</td>
<td>0.98 (0.95, 1.01)</td>
<td>0.3</td>
</tr>
<tr>
<td>Education* (11180, 14329, 3623)</td>
<td>38.5 (37.56, 39.45)</td>
<td>38.37 (37.57, 39.17)</td>
<td>38.15 (36.77, 39.54)</td>
<td>1.00 (0.97, 1.04)</td>
<td>0.8</td>
</tr>
<tr>
<td>Income* (4998, 7070, 2377)</td>
<td>49.35 (48.37, 50.32)</td>
<td>50.32 (49.5, 51.15)</td>
<td>51.00 (49.56, 52.43)</td>
<td>0.96 (0.92, 0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Proportion (95% CI)</td>
<td>OR (95% CI)</td>
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<tr>
<td><strong>MC4R genotypes (major homozygote/heterozygote/minor homozygote)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(18005/37027)</td>
<td>48.37 (47.7, 49.05)</td>
<td>48.41 (46.37, 50.46)</td>
<td>1.02 (0.98, 1.05)</td>
<td>0.4</td>
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</tr>
<tr>
<td><strong>Drinking</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(21117/37027)</td>
<td>57.1 (56.43, 57.77)</td>
<td>55.98 (53.94, 58)</td>
<td>0.99 (0.96, 1.03)</td>
<td>0.6</td>
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</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(11180, 14329, 3623)</td>
<td>38.39 (37.65, 39.14)</td>
<td>38.77 (36.53, 41.05)</td>
<td>1.01 (0.97, 1.05)</td>
<td>0.7</td>
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</tr>
<tr>
<td><strong>Income</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(8192, 5334, 919)</td>
<td>50.05 (49.29, 50.82)</td>
<td>49.85 (49.56, 54.21)</td>
<td>1.00 (0.96, 1.05)</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

Proportion (95% CI) by tertile of body mass index and FTO/MC4R genotype.
* indicates tripartite categorical variable (other variables are binary). Proportion in lowest group for education is shown (0 = 0-9 years; 1 = 10-12 years; 2 = 13 years).
For income, proportion in lowest bracket are again shown (0 < 400,000 Kr; 1 = 400,000 - 600,000 Kr; 2 > 600,000 Kr)
OR (95% CI) indicates a logistic regression derived odds ratio for the per unit effect of either body mass index tertile, FTO genotype or MC4R genotype on unit of confounder (p is a p-value for this test). Effect adjusted for age and sex.
Figure S1.

Relationship of systolic and diastolic blood pressure with body mass index in the Copenhagen General Population Study cohort.

Blood pressure (mmHg)

Correlation coefficients for log relative body mass index (BMI) and systolic (diastolic) blood pressure are 0.20 and 0.24 respectively (p<0.0001).

Green squares indicate a scatter plot of systolic blood pressure by relative BMI.
Orange circles indicate a scatter plot of diastolic blood pressure by relative BMI.
Lines show a local linear regression (bandwidth 0.1) between blood pressure measures and relative BMI.