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Original article

Causal relationship between dietary macronutrient composition and anthropometric measures: A bidirectional two-sample Mendelian randomization analysis

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SUMMARY

Background: The question whether the proportion of energy provided by fat and carbohydrates in the diet is associated with body mass index (BMI) and waist circumference (WC) is an important public health issue, but determining causality is difficult in epidemiological studies.

Objectives: Using a two-sample bidirectional Mendelian randomization (MR) in both a univariable and multivariable setting, we aimed to determine whether the relative proportion of different macronutrients in the diet (in % of total energy intake (E%)) is causally related to BMI and WC and vice versa.

Methods: All analyses were based on genome-wide association studies including 268,922 Europeans with dietary data (SSGAC Consortium) and at least 232,101 with anthropometric measures (GIANT Consortium). An inverse-variance weighted approach using modified second-order weights within the radial regression framework was performed. Radial MR-Egger, weighted median and mode, Robust Adjusted Profile Score (RAPS), and Pleiotropy RESidual Sum and Outlier (PRESSO) methods were used in sensitivity analyses to verify MR assumptions. Additionally, multivariable MR was conducted to account for inter correlation between macronutrient intakes. All estimates represent the standard deviation (SD) change in each outcome per one SD change in the respective exposure.

Results: We found that genetically predicted relative carbohydrate intake (E%) reduced BMI ($\beta = -0.529$; 95% CI: −0.745, −0.312; P-value = $2{\cdot}10^{-6}$) and WC ($\beta = -0.459$; 95% CI: −0.656, −0.262; P-value = $5{\cdot}10^{-6}$). Both effects were also supported by the multivariable approach: $\beta = -0.441$ (95% CI: −0.772, −0.109; P-value = 0.009) for BMI and $\beta = -0.410$ (95% CI: −0.667, −0.154; P-value = 0.002) for WC. Genetically predicted dietary intake of fat (E%) was weaker and positively related to both anthropometric measures. We obtained evidence that a higher BMI and WC increased the relative dietary intake of fat and protein (E%). For example, each SD higher BMI increased protein intake (E%) by 0.114 SD (95% CI: 0.081, 0.147; P-value = $9{\cdot}10^{-12}$) and each SD higher WC increased protein intake (E%) by 0.078 SD (95% CI: 0.035, 0.121; P-value = $4{\cdot}10^{-4}$). Sensitivity analyses confirmed these findings revealing consistent effect estimates.

Conclusions: Using genetic information to improve causal inference we found evidence, that a low relative carbohydrate proportion (E%) and a high proportion of fat (E%) in the diet is causally related to a higher BMI and a higher WC.

Further research considering carbohydrate, fat, and protein quality and possible consequences on micronutrient intake is needed to define the implications for dietary intake recommendations.

1. Introduction

Worldwide, obesity has almost tripled since 1975 and is now regarded as one of the most urgent public health threats, reaching epidemic proportions. In 2016, an estimated 650 million people

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worldwide were obese [1] and it is predicted that this number will increase to 1.12 billion by 2030 [2]. Obesity can be quantified by anthropometric measurements such as body mass index (BMI) calculated as the ratio of body weight by squared body height $\left(\frac{kg}{m^2}\right)$ and waist circumference (WC) in cm. These measures are commonly used to estimate (excess) body fat and the likelihood to develop weight-related diseases, such as diabetes mellitus, hypertension, some forms of cancer, coronary heart disease, and osteoarthritis [1]. According to the World Health Organization obesity is defined with a BMI $\geq$ 30 $\frac{kg}{m^2}$ and a WC $\geq$ 88 cm in women and 102 cm in men reflects abdominal body fat accumulation that substantially increases the risk of metabolic complications [1].

To gain weight, energy intake has to exceed energy expenditure [3]. However, body weight regulation is complex and depends on interactions between metabolic, genetic, environmental and psychosocial factors [4,5]. While physical activity has decreased over the past decades, energy intake has not decreased in the same way, and the relative proportion of energy from macronutrients, i.e. carbohydrates, fat, and protein has also changed over time.

The composition of the diet appears to play an important role in the regulation of body weight; in particular, the contribution of fat and carbohydrates to total energy intake seems to be relevant in this context. Observational studies, which are prone to reverse causality and residual confounding, have reported inconsistent results regarding the effects of macronutrient composition on health [6,7]. In addition, there is little evidence from randomized controlled trials on the long-term impact of specific macronutrient restriction on body weight and health [8,9]. Nevertheless, a number of different diets for weight loss have been popularized in the last years, including high-carbohydrate/low-fat and low-carbohydrate/high fat diets; so far, there is no clear consensus on what macronutrient ratio is optimal for obesity prevention and treatment.

A Mendelian randomization (MR) approach using genetic variants as instrumental variables offers the opportunity to identify causal relationships in the presence of unobserved confounding and reverse causation [10]. In this study, we performed a bidirectional two-sample MR analysis to provide evidence for a causal effect between the relative intake of fat, carbohydrates, and proteins and the anthropometric measures BMI and WC.

2. Methods

2.1. Study design

In a bidirectional two-sample MR approach, genetic variants were used to assess the causal impact and direction of relative dietary carbohydrate, fat, and protein intake with BMI and WC as measures of overall and abdominal body fat. Briefly, single nucleotide polymorphisms (SNPs) used as instruments for modifiable risk factors are randomly allocated at conception and are therefore less likely to be affected by confounding or reverse causation. Regarding natural randomization, the MR approach mimics a randomized controlled trial based on individual or summary level data from observational studies. However, for reliable results, the key assumptions of an instrumental variable (IV) setting must be ensured, i.e. IVs must be associated with the exposure, not related to confounders of the exposure-outcome association, and affect the outcome only through the exposure (Fig. 1). We extended this design in a multivariable setting, investigating the simultaneous impact of the intake of all three correlated macronutrients (in % of total energy intake (E%)) on anthropometric measures (Fig. 2). Details on the MR-design have been described elsewhere [10–12].

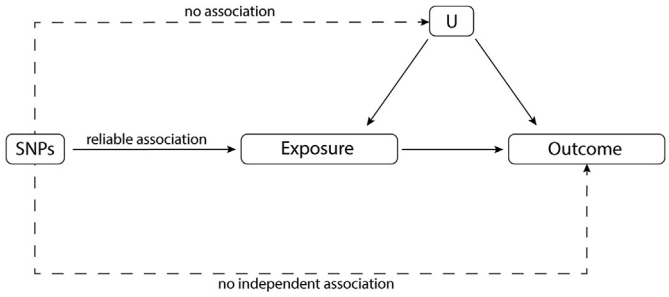

Fig. 1. Illustration of the general Mendelian randomization setting with required instrumental variable assumptions (according to Holmes et al. [53]). That is, each genetic variant (SNP) is (i) associated with the exposure, (ii) not associated with unmeasured confounders (U) of the exposure-outcome association, and (iii) not associated with the outcome conditional on the exposure.

For each modelling direction, the study had three main components: acquisition of suitable genetic instruments for the appropriate exposure, application of several MR methods, and sensitivity analyses as described below.

2.2. Diet composition cohort

For our study, summary statistics of genome-wide association studies (GWASs) from Meddens et al. [13] were derived from the Social Science Genetic Association Consortium and include 268,922 individuals (Supplementary Fig. 1). The sample consists of 83% Europeans, 60% women, and an age range between 27 and 71 years. Findings from the discovery GWAS analysis in the UK Biobank (n = 175,253) were replicated by Meddens et al. in 14 studies including ALSPAC, DietGen, EPIC-InterAct1 and 2, Fenland, Framingham Heart Study, Health and Retirement Study, LifeLines, Rotterdam Studies 1, 2, and 3, and Women's Health Initiative. A meta-analysis of GWAS summary statistics from these cohorts (n = 60,138) together with summary statistics from DietGen (n = 33,531) was conducted by Meddens et al. in the replication phase. In the included studies, habitual dietary intake data obtained by self-reported food frequency questionnaires and at least 70 food items were used to calculate the relative intake of the three macronutrients, carbohydrates, fat, and protein (Supplementary Table 1). An exception was the UK Biobank diet data, which is based on single 24-h diet recalls. Across cohorts, average intakes were highly similar. All cohorts (except DietGen) expressed macronutrient intakes in % of total energy intake (E%) (for details see Meddens et al. [13]).

2.3. Anthropometric measurements cohorts

In our analysis, we used two different anthropometric measures, namely BMI and WC. For both measures, the GWASs summary statistics of Locke et al. (n = 322,154) and Shungin et al. (n = 232,101) were used, respectively (Supplementary Fig. 1). These two studies arose from the GIANT consortium that contains subjects of European ancestry, aged 12 to 107 and 12–113 years, and with 51% and 55% women, respectively. Summary-level data for BMI consist of 125 studies (82 from GWASs and 43 from Metabochip studies) and for WC of 101 studies (57 from GWASs and 44 from Metabochip studies). In all original studies, ethics approval had been obtained for all participants.

2.4. Sample overlap

There were five partially overlapping studies (Fenland, HRS, LifeLines, RS-I, and RS-III) of macronutrient composition and BMI

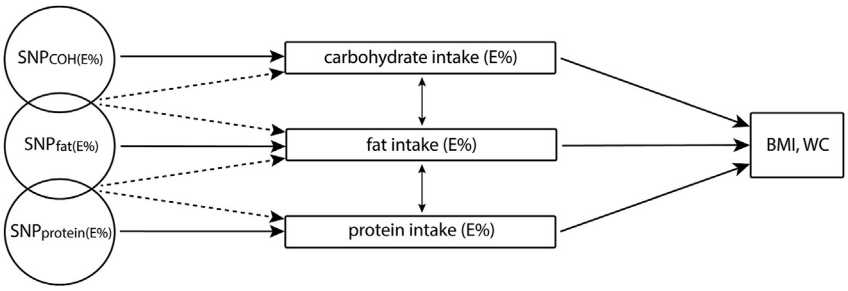

Fig. 2. Simplified illustration of the multivariable Mendelian randomization setting of the correlated relative dietary intake of macronutrients (in % of total energy intake (E%)) on the anthropometric measures BMI and WC. Abbreviations: $SNP_{COH(E\%)}$, single nucleotide polymorphisms associated with carbohydrate intake (E%); $SNP_{fat(E\%)}$, single nucleotide polymorphisms associated with fat intake (E%); $SNP_{protein(E\%)}$, single nucleotide polymorphisms associated with protein intake (E%).

GWASs (Supplementary Table 2). Hence, the proportion of sample overlap across all studies was at most 6.4% for dietary intakes (E%) and 5.4% for BMI. WC GWAS was also partially overlapping in five studies (Fenland, LifeLines, RS-I, RS-II, and RS-III) with the macronutrient composition GWAS resulting in an overlap proportion of at most 6.9% (WC GWAS) and 5.9% (macronutrient composition GWAS), respectively. To quantify the impact of sample overlaps we calculated the magnitude of expected bias according to Burgess et al. [14]. All results were below a value of $6.8 \cdot 10^{-4}$ indicating almost unbiased effect estimates due to sample overlaps (Supplementary Table 3).

2.5. Selection of genetic instruments

As independent instruments we considered genetic variants that met the following criteria: First, SNPs should be associated with the appropriate exposure at the genome-wide significance threshold $P = 5 \cdot 10^{-8}$. Second, SNPs should be uncorrelated, i.e. not in linkage disequilibrium (LD) using a stringent clumping algorithm with cut-off $r^2 = 0.001$ in 10,000 Kb windows. Third, SNPs should not be palindromic with intermediate allele frequencies between 0.43 and 0.57. Therefore, 68 BMI, 41 WC, 2 fat, 5 protein, and 11 carbohydrate related independent SNPs were considered as instruments for main MR analyses (Supplementary Table 4). Differences in the number of SNPs extracted by Locke et al. were due to the more stringent settings used as default by the clumping algorithm in the present study.

Regarding weak instruments, we calculated SNP-specific F-statistics as a measure of instrument strength (Supplementary Tables 5–9). The lowest F-statistics ranged between 29.02 (BMI) and 32.04 (relative protein intake) and were therefore above 10, indicating generally strong instruments and therefore low predisposition for weak instrument bias (Supplementary Table 4).

Some of the SNPs were strongly related to possible confounding factors (labeled as U in Fig. 1) of the relationship between dietary macronutrient composition and anthropometric measures (i.e. alcohol consumption, smoking status, physical activity, education, and depressive symptoms [15–17]) based on the genome-wide significance threshold of $5 \cdot 10^{-8}$. Identified by a PhenoScanner search, we excluded these SNPs as part of a sensitivity analysis to prevent a possible effect of the genetic variants through the confounder on the outcome, known as horizontal pleiotropy (Supplementary Table 10), and used finally 56 BMI-, 32 WC-, 2 fat-, 3 protein-, and 5 carbohydrate-related genetic variants (Supplementary Table 4). To meet the assumption that requires instruments to be associated with the outcome only through the exposure, we excluded all SNPs or proxy-SNPs in linkage disequilibrium that are strongly associated with the respective outcome. Estimates of instrument-outcome relationships based on the remaining SNPs can be found in Supplementary Tables 11–15.

2.6. Statistical analyses

2.6.1. Bidirectional MR

With respect to the different sample sizes, pooled means and standard deviations (SDs) were calculated across all studies used in the corresponding GWAS. Causal effect estimates of bidirectional relationships between macronutrient intake (E%) and anthropometric measures were obtained, applying an inverse-variance weighted (IVW) approach using modified second-order weights within the radial regression framework. This method provides the highest statistical power but requires the validity of the MR key assumptions.

The small number of instruments in case of relative fat and protein intake (with two and five SNPs, respectively) led to problems within heterogeneity analysis as well as outlier assessment. Beyond that, several issues (e.g. too few SNPs to undertake the analyses, abnormally large overdispersion and convergence problems of optimization algorithms) resulted in missing estimates for the most MR-approaches. To overcome these issues and verify the results for relative fat and protein intake we relaxed the GWAS threshold to $P = 5 \cdot 10^{-6}$ for these macronutrients. Consequently, in a sensitivity analysis 24 fat- and protein-related independent SNPs were considered hereafter as instruments for MR analyses (Supplementary Table 16).

In further sensitivity analyses, the MR key assumptions were validated by implementing a series of robust methods within sensitivity analyses that consider different patterns of heterogeneity. Initially, the weighted median approach was performed, which provides consistent estimates if at least 50% of the exposure-associated genetic variants represent valid instruments. For the sake of consistency, we conducted a weighted mode regression that allows more than 50% of the genetic variants to be invalid. The radial MR-Egger approach was applied to estimate causal effects even in the case of directional pleiotropy. Fourth, within a many weak instruments analysis we used the Robust Adjusted Profile Score (RAPS) method while controlling for overdispersion as an indicator for systematic pleiotropy. Beyond that, the MR-PRESSO (Pleiotropy RESidual Sum and Outlier) analysis was performed for two issues: the global test detected potential horizontal pleiotropy, and the outlier test identified influential genetic instruments at a threshold of $\alpha = 0.05$ (Supplementary Table 17).

Directional pleiotropy was evaluated by the MR-Egger intercept test and substantial heterogeneity within the IVW and MR-Egger methods was quantified and tested using Cochran's as well as Rücker's Q statistics (Supplementary Table 17). Outcome-associated instruments were dropped and influential SNPs were identified and

excluded with respect to the appropriate Q statistic (Supplementary Tables 18–22) and visual assessment of radial, funnel, leave-one-out, and MR-scatter plots.

2.6.2. Statistical power

Given a type I error of 5%, statistical power was calculated for each causal effect estimate and additionally for confidence intervals (CI) (obtained from the radial IVW approach with modified second-order weights) according to Brion et al. [18]. In this way, our power analysis covered the most probable range of estimates for the underlying true causal effect. Assuming a conservative observational estimate of 0.3 (i.e. stronger power for larger observational estimates) our MR analyses were sufficiently powered especially in the direction of dietary composition on anthropometric measures with a statistical power $\geq$ 0.98 for point estimates (Supplementary Table 3). However, in the models of BMI and WC on carbohydrate intake (E%) the power was 0.13 and 0.12, respectively, for resulted point estimates.

2.6.3. Multivariable MR

Obviously, the relative intakes of the macronutrients relate to each other, and as a result, there were SNPs that were associated with at least two of the three macronutrients. Considering these relationships, we finally performed a multivariable MR analysis to determine effect estimates of each macronutrient conditioned on one another. Regarding a threshold of $P = 5{\cdot}10^{-8}$, 13 and 14 genetic variants could be considered in the analyses with BMI and WC, respectively, after excluding outcome-associated SNPs (Supplementary Table 23). We applied the multivariable IVW regression with multiplicative random effects. Within sensitivity analyses, causal estimates were determined by the MR-Egger, Median and Q-minimization approaches. The latter method, returning causal point estimates, account for both the weak instruments and the substantial heterogeneity that were quantified by global Q-statistics and conditional F-statistics, respectively (Supplementary Table 24). Like in the univariable case, MR-Egger intercept tests were used with the multivariable models containing all three macronutrients at once.

To account for multiple testing, the Bonferroni correction was applied to the two sets of variables (two anthropometric measures and three macronutrients) that were tested against each other. Thus, we calculated the conservative corrected threshold by dividing 0.05 by the six investigated null hypotheses, and considered P-values < 0.008 as strongly associated and values of $0.008 \leq P < 0.05$ as suggestively associated. All reported estimates were expressed as SD change in an outcome per one SD increment of the exposure. Substantial heterogeneity of a specific SNP was assumed, if the probability of the respective Q statistic were below the threshold of $\alpha_Q = 0.01$. The decision rules for all other statistical tests based on an $\alpha = 0.05$. Analyses were performed using primarily the TwoSampleMR (version 0.5.4), MendelianRandomization (version 0.4.2), and MRPRESSO (version 1.0) packages of the statistical Software R (version: 4.0.0).

3. Results

3.1. Descriptive statistics

Across all cohorts, total energy intake was on average 2064 kcal (SD = 609.9) and was composed of 49 E% carbohydrates, 35 E% fat, and 16 E% proteins. The mean BMI calculated across the studies used in the anthropometric measures GWASs was 26.8 $\frac{kg}{m^2}$ (SD = 4.7 $\frac{kg}{m^2}$) and WC 90.1 cm (SD = 12.5 cm). In the main analyses, the 68 genetic instruments explained 1.25% of the variance in BMI. Regarding WC, 0.89% of the variance was explained by the 41 instrumental SNPs. The 2 fat intake (E%) related, 5 protein intake (E%) related, and 11 carbohydrate intake (E%) related genetic instruments explained 0.06%, 0.12%, and 0.17% of the variance, respectively.

3.2. Causal effects between macronutrient composition and anthropometric measures

3.2.1. Main analyses

Regarding the Radial IVW models with modified second-order weights (point estimates hereafter referred to as β_{IVW}), we observed a strongly inverse effect of genetically predicted carbohydrate intake (E%) on change in both anthropometric measures (for BMI: $\beta_{IVW} = -0.529$ per 1 SD; 95% CI: −0.745, −0.312; P-value $= 2{\cdot}10^{-6}$ and for WC: $\beta_{IVW} = -0.459$ per 1 SD; 95% CI: −0.656, −0.262; P-value $= 5{\cdot}10^{-6}$). However, a causal effect in the reverse direction could not be confirmed due to low power. The effect estimates of BMI and WC on SD change in relative carbohydrate intake were $\beta_{IVW} = -0.014$ per 1 SD (95% CI: −0.050, 0.022; P-value = 0.451) and $\beta_{IVW} = -0.016$ per 1 SD (95% CI: −0.063, 0.031; P-value = 0.502), respectively (Tables 1 and 2, Figs. 3 and 4, Supplementary Table 3).

Additionally, there was evidence supporting bidirectional positive effects between the relative proportion of fat intake with BMI as well as WC (Figs. 3 and 4). Although estimates for the effect of relative fat intake on both anthropometric measures could not be obtained by the Radial IVW models with modified second-order weights due to numerical issues (e.g. abnormally large overdispersion, errors due to convergence problems of optimization algorithms, and too few instruments to undertake the analyses), the IVW approach with multiplicative random effects revealed strong effects in the positive direction. Thus, the effect estimate of relative fat intake on BMI was $\beta = 0.299$ (95% CI: 0.285, 0.313; P-value $= 1{\cdot}10^{-47}$) and on WC $\beta = 0.238$ (95% CI: 0.201, 0.275; P-value $= 2{\cdot}10^{-36}$) (Table 1). In the reverse direction, the effect estimates of BMI and WC on SD change in relative fat intake were $\beta_{IVW} = 0.039$ per 1 SD (95% CI: 0.009, 0.070; P-value = 0.012) and $\beta_{IVW} = 0.065$ per 1 SD (95% CI: 0.020, 0.011; P-value = 0.005), respectively (Table 2, Fig. 4).

Regarding the relative intake of proteins, (where calculation was possible) inconsistent effects on SD change in both anthropometric measures were found.. The effect estimates on a SD change in protein intake (E%) were $\beta_{IVW} = 0.114$ per 1 SD in BMI (95% CI: 0.081, 0.147; P-value $= 9{\cdot}10^{-12}$) and $\beta_{IVW} = 0.078$ per 1 SD in WC (95% CI: 0.035, 0.121; P-value $= 4{\cdot}10^{-4}$). In the reverse direction the effect estimates of BMI and WC on SD change in relative protein intake were $\beta_{IVW} = 0.114$ per 1 SD (95% CI: 0.081, 0.147; P-value $= 9{\cdot}10^{-12}$) and $\beta_{IVW} = 0.078$ per 1 SD (95% CI: 0.035, 0.121; P-value $= 4{\cdot}10^{-4}$), respectively (Table 2, Fig. 4).

3.2.2. Sensitivity analyses

To verify the results of the main analysis, we conducted a sensitivity analysis using a relaxed GWAS threshold of $P = 5{\cdot}10^{-6}$ for relative fat and protein intake. The causal effect estimates of fat intake (E%) on SD change in BMI ($\beta_{IVW} = 0.169$ per 1 SD; 95% CI: 0.035, 0.302; P-value = 0.013) and WC ($\beta_{IVW} = 0.239$ per 1 SD; 95% CI: 0.042, 0.435; P-value = 0.017) were similar as for the stronger threshold (Table 3, Fig. 5). Furthermore, we observed a strong positive effect of genetically predicted protein intake on both anthropometric measures, with $\beta_{IVW} = 0.125$ SD change in BMI (95% CI: 0.013, 0.237; P-value = 0.028) and $\beta_{IVW} = 0.169$ SD change in WC (95% CI: 0.060 0.279; P-value = 0.002) per one SD change in protein intake (E%).

Table 1
Mendelian randomization estimates (standard deviation (SD) change in each outcome per one SD change in the respective exposure) for the causal effect of genetically predicted relative dietary intake of carbohydrates, fat, and proteins (in % of total energy intake (E%)) on the anthropometric measures body mass index (BMI) and waist circumference (WC) based on the genome-wide significance threshold $P = 5 \cdot 10^{-8}$.

Method	n_{SNP}	β	95% CI	P-value
Carbohydrate intake (E%) → BMI[a,b]				
IVW (mult. random effects)	8	−0.526	(−0.742, −0.310)	2E−06
Radial MR-Egger	8	−0.207	(−1.183, 1.596)	0.780
Weighted median	8	−0.389	(−0.627, −0.151)	0.001
Weighted mode	8	−0.316	(−0.622, −0.010)	0.082
Radial IVW (Mod.2nd)	8	−0.529	(−0.745, −0.312)	2E−06
Radial IVW (exact fixed effects)	8	−0.549	(−0.716, −0.383)	1E−10
Radial IVW (exact random effects)	8	−0.539	(−0.743, −0.336)	1E−03
RAPS	8	−0.555	(−0.76, −0.349)	1E−07
PRESSO	8	−0.526	(−0.742, −0.310)	0.002
Carbohydrate intake (E%) → WC				
IVW (mult. random effects)	10	−0.460	(−0.657, −0.263)	5E−06
Radial MR-Egger	10	−0.638	(−1.833, 0.556)	0.326
Weighted median	10	−0.394	(−0.614, −0.175)	4E−04
Weighted mode	10	−0.371	(−0.739, −0.003)	0.080
Radial IVW (Mod.2nd)	10	−0.459	(−0.656, −0.262)	5E−06
Radial IVW (exact fixed effects)	10	−0.477	(−0.635, −0.319)	3E−09
Radial IVW (exact random effects)	10	−0.471	(−0.664, −0.277)	0.001
RAPS	10	−0.464	(−0.671, −0.256)	1E−05
PRESSO	10	−0.460	(−0.657, −0.263)	0.001
Fat intake (E%) → BMI				
IVW (mult. random effects)	2	0.299	(0.285, 0.313)	1E−47
RAPS	2	0.299	(0.066, 0.532)	0.012
Fat intake (E%) → WC				
IVW (mult. random effects)	2	0.238	(0.201, 0.275)	2E−36
RAPS	2	0.238	(−0.035, 0.511)	0.087
Protein intake (E%) → BMI				
IVW (mult. random effects)	5	−0.046	(−0.380, 0.287)	0.0786
Radial MR-Egger	5	1.191	(−0.148, 2.531)	0.180
Weighted median	5	−0.005	(−0.225, 0.215)	0.964
Weighted mode	5	−0.034	(−0.342, 0.274)	0.838
RAPS	5	−0.005	(−0.256, 0.246)	0.970
PRESSO	5	−0.050	(−0.140, 0.039)	0.387
Protein intake (E%) → WC				
IVW (mult. random effects)	5	0.034	(−0.288, 0.356)	0.837
Radial MR-Egger	5	1.280	(−0.016, 2.575)	0.148
Weighted median	5	0.105	(−0.135, 0.344)	0.393
Weighted mode	5	0.265	(−0.093, 0.623)	0.220
Radial IVW (Mod.2nd)	5	0.034	(−0.288, 0.356)	0.837
Radial IVW (exact fixed effects)	5	0.036	(−0.136, 0.208)	0.682
Radial IVW (exact random effects)	5	0.034	(−0.283, 0.352)	0.842
RAPS	5	0.084	(−0.180, 0.348)	0.532
PRESSO	5	0.158	(−0.106, 0.421)	0.325

Abbreviations: IVW (mult. random effects), inverse-variance weighted (multiplicative random effects); Mod.2nd, modified second-order weights; RAPS, Robust Adjusted Profile Score; PRESSO, Pleiotropy RESidual Sum and Outlier.
[a] Possible pleiotropy revealed by MR PRESSO global test.
[b] Possible horizontal pleiotropy revealed by Cochran's Q-statistic.

We further conducted a series of sensitivity analyses to assess the robustness of the results. After exclusion of influential genetic variants based on Cochran's and Rücker's SNP-specific Q_j statistics in an iterative procedure (Supplementary Tables 18–22), the robust MR-methods emphasized largely the findings of the IVW models (Tables 1–3, Figs. 3–5). While RAPS, PRESSO and the weighted median estimates were consistent throughout regarding strongly associated results, the weighted mode approach differed slightly for the effect of BMI on fat intake (E%) as well as in the case of protein intake (E%) with WC. The same applied for radial MR-Egger estimate of the impact of relative carbohydrate intake on BMI. Due to low −power there was only a weak causal effect of both anthropometric measures on carbohydrate intake (E%).

Furthermore, regarding the global MR-PRESSO together with Cochran's Q tests, three of the final models exhibited horizontal but balanced pleiotropy, since the appropriate radial MR-Egger intercepts ranged between $\beta_0 = 0.17$; SE = 2.85; P-value = 0.955 (carbohydrate intake (E%) on BMI) and $\beta_0 = 0.89$; SE = 0.87; P-value = 0.313 (WC on fat intake (E%)) regarding the deviation from zero (Supplementary Table 17). Under the "Instrument Strength Independent of Direct Effect" (InSIDE) assumption, heterogeneity due to horizontal pleiotropy in these cases did not automatically invalidate the respective IVW estimates; this is why the switch to random effects IVW models resulted in equivalent estimates compared to models with fixed effects (Figs. 3 and 4). However, due to the MR-Egger intercept tests as well as the test of $Q - Q' \sim \chi^2_1$ statistics with respect to the ratio $Q_R = \frac{Q'}{Q}$, three models seemed to have directional pleiotropy (Supplementary Table 17). In these cases the radial MR-Egger analysis provided again a consistent estimate but with comparatively large CI for the notable impact of relative fat intake on WC and, as before, inconsistent estimates for the impact of both anthropometric measures on carbohydrate intake (E%) (Figs. 4 and 5).

To summarize, except for weak associated effect estimates, the switch to models suggested by heterogeneity statistics and accounting for the appropriate pattern of pleiotropy also led to consistent estimates compared to the initial radial IVW approach

Table 2
Mendelian randomization estimates (standard deviation (SD) change in each outcome per one SD change in the respective exposure) for the causal effect of genetically predicted body mass index (BMI) and waist circumference (WC) on relative dietary intake of carbohydrates, fat, and proteins (in % of total energy intake (E%)) based on the genome-wide significance threshold $P = 5 \cdot 10^{-8}$.

Method	n_{SNP}	β	95% CI	P-value
BMI → Carbohydrate intake (E%)[a,b,c,e]				
IVW (mult. random effects)	57	−0.014	(−0.050, 0.022)	0.451
Radial MR-Egger	57	0.115	(−0.003, 0.233)	0.061
Weighted median	57	0.014	(−0.033, 0.062)	0.556
Weighted mode	57	0.031	(−0.047, 0.109)	0.438
Radial IVW (Mod.2nd)	57	−0.014	(−0.050, 0.022)	0.451
Radial IVW (exact fixed effects)	57	−0.014	(−0.045, 0.017)	0.365
Radial IVW (exact random effects)	57	−0.014	(−0.053, 0.025)	0.484
RAPS	57	−0.016	(−0.053, 0.021)	0.393
PRESSO	57	−0.014	(−0.050, 0.022)	0.454
BMI → Fat intake (E%)				
IVW (mult. random effects)	63	0.039	(0.009, 0.070)	0.012
Radial MR-Egger	63	0.031	(−0.084, 0.147)	0.597
Weighted median	63	0.023	(−0.023, 0.070)	0.320
Weighted mode	63	−0.010	(−0.095, 0.074)	0.814
Radial IVW (Mod.2nd)	63	0.039	(0.009, 0.070)	0.012
Radial IVW (exact fixed effects)	63	0.040	(0.010, 0.070)	0.009
Radial IVW (exact random effects)	63	0.040	(0.010, 0.070)	0.012
RAPS	63	0.040	(0.009, 0.071)	0.011
PRESSO	63	0.039	(0.009, 0.070)	0.015
BMI → Protein intake (E%)				
IVW (mult. random effects)	63	0.114	(0.081, 0.147)	9E−12
Radial MR-Egger	63	0.038	(−0.073, 0.149)	0.503
Weighted median	63	0.086	(0.043, 0.130)	3E−04
Weighted mode	63	0.052	(−0.026, 0.131)	0.197
Radial IVW (Mod.2nd)	63	0.114	(0.081, 0.147)	9E−12
Radial IVW (exact fixed effects)	63	0.116	(0.087, 0.145)	4E−15
Radial IVW (exact random effects)	63	0.116	(0.085, 0.147)	4E−10
RAPS	63	0.115	(0.082, 0.148)	7E−12
PRESSO	63	0.114	(0.081, 0.147)	4E−09
WC → Carbohydrate intake (E%)[a,c,e]				
IVW (mult. random effects)	37	−0.016	(−0.063, 0.031)	0.502
Radial MR-Egger	37	0.160	(−0.024, 0.344)	0.098
Weighted median	37	0.013	(−0.045, 0.071)	0.666
Weighted mode	37	0.066	(−0.021, 0.154)	0.145
Radial IVW (Mod.2nd)	37	−0.016	(−0.063, 0.031)	0.502
Radial IVW (exact fixed effects)	37	−0.017	(−0.054, 0.021)	0.389
Radial IVW (exact random effects)	37	−0.016	(−0.063, 0.030)	0.493
RAPS	37	−0.017	(−0.064, 0.029)	0.470
PRESSO	37	−0.016	(−0.063, 0.031)	0.507
WC → Fat intake (E%)[a,c]				
IVW (mult. random effects)	38	0.065	(0.020, 0.110)	0.005
Radial MR-Egger	38	−0.037	(−0.239, 0.164)	0.717
Weighted median	38	0.050	(−0.007, 0.107)	0.083
Weighted mode	38	0.015	(−0.082, 0.112)	0.761
Radial IVW (Mod.2nd)	38	0.065	(0.020, 0.110)	0.005
Radial IVW (exact fixed effects)	38	0.067	(0.029, 0.105)	0.001
Radial IVW (exact random effects)	38	0.066	(0.023, 0.109)	0.005
RAPS	38	0.065	(0.020, 0.110)	0.005
PRESSO	38	0.065	(0.020, 0.110)	0.008
WC → Protein intake (E%)[a,c,d]				
IVW (mult. random effects)	41	0.078	(0.035, 0.121)	4E−04
Radial MR-Egger	41	0.160	(−0.024, 0.343)	0.096
Weighted median	41	0.064	(0.007, 0.120)	0.026
Weighted mode	41	0.021	(−0.093, 0.136)	0.715
Radial IVW (Mod.2nd)	41	0.078	(0.035, 0.121)	4E−04
Radial IVW (exact fixed effects)	41	0.080	(0.045, 0.116)	9E−06
Radial IVW (exact random effects)	41	0.080	(0.035, 0.124)	0.001
RAPS	41	0.078	(0.035, 0.121)	4E−04
PRESSO	41	0.078	(0.035, 0.121)	0.001

Abbreviations: IVW, inverse-variance weighted; Mod.2nd, modified second-order weights; RAPS, Robust Adjusted Profile Score; PRESSO, Pleiotropy RESidual Sum and Outlier.
[a] Possible pleiotropy revealed by MR PRESSO global test.
[b] Directional pleiotropy revealed by Radial MR-Egger intercept test.
[c] Possible horizontal pleiotropy revealed by Cochran's Q-statistic.
[d] Possible heterogeneity about Egger revealed by Rücker's Q′-statistic.
[e] Suggested MR-Egger over the IVW method by difference of Q–Q′ and ratio Q′/Q.

and therefore did not alter our findings. Beyond that, the strength of the chosen instruments quantified by F-statistics with means between 28.34 (fat intake) and 57.52 (BMI) (Supplementary Tables 4 and 16) indicated absence of weak instrument bias, especially with regard to instrument selections for the relative proportion of fat and protein intake with the relaxed threshold.

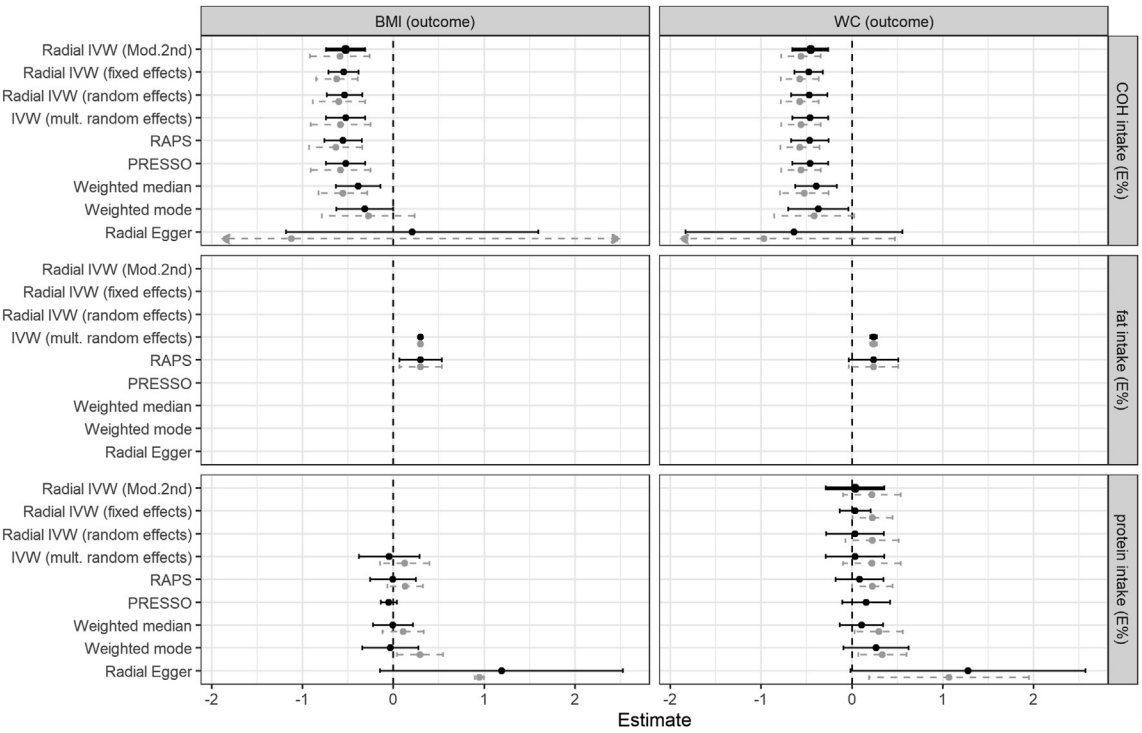

Fig. 3. Causal estimates and 95% confidence intervals (standard deviation (SD) change in each outcome per one SD change in the respective exposure) of the impact of relative dietary intake of carbohydrates (COH), fat, and proteins (in % of total energy intake (E%)) on body mass index (BMI) and waist circumference (WC) based on the genome-wide significance threshold $P = 5 \cdot 10^{-8}$. Bold lines illustrate the main analyses. Grey points with dashed confidence intervals represent effects of sensitivity analyses after exclusion of potential confounder-associated single nucleotide polymorphisms (SNPs) due to control of horizontal pleiotropy. Arrows indicate confidence intervals exceeding the plot range. Missing estimates are due to numerical issues within the appropriate algorithms (e.g. abnormally large overdispersion, errors due to convergence problems of optimization algorithms, and too few instruments to undertake the analyses). Abbreviations: IVW (mult. random effects), inverse-variance weighted (multiplicative random effects); Mod.2nd, modified second-order weights; RAPS, Robust Adjusted Profile Score; PRESSO, Pleiotropy RESidual Sum and Outlier.

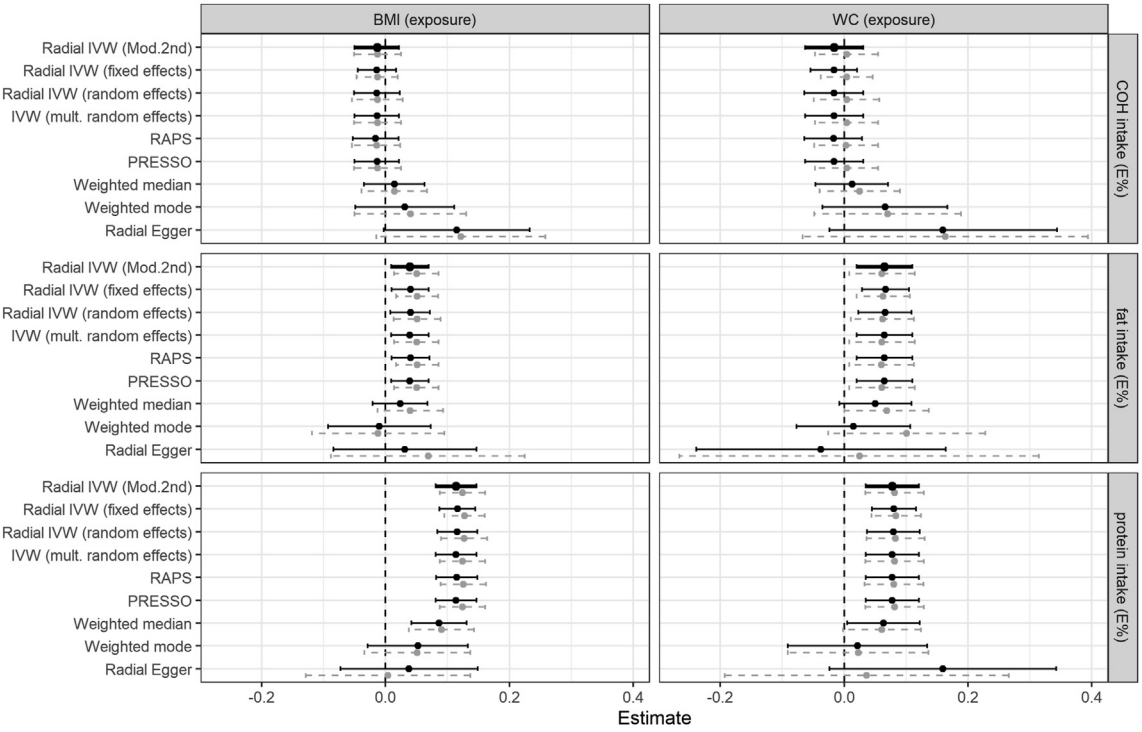

Fig. 4. Causal estimates and 95% confidence intervals (standard deviation (SD) change in each outcome per one SD change in the respective exposure) of the impact of body mass index (BMI) and waist circumference (WC) on relative dietary intake of carbohydrates (COH), fat, and proteins (in % of total energy intake (E%)). Bold lines illustrate the main analyses. Grey points with dashed confidence intervals represent effects of sensitivity analyses after exclusion of potential confounder-associated single nucleotide polymorphisms (SNPs) due to control of horizontal pleiotropy. Abbreviations: IVW (mult. random effects), inverse-variance weighted (multiplicative random effects); Mod.2nd, modified second-order weights; RAPS, Robust Adjusted Profile Score; PRESSO, Pleiotropy RESidual Sum and Outlier.

Table 3
Sensitivity analysis: Mendelian randomization estimates (standard deviation (SD) change in each outcome per one SD change in the appropriate exposure) for the causal effect of genetically predicted relative dietary intake of fat, and proteins (in % of total energy intake (E%)) on the anthropometric measures body mass index (BMI) and waist circumference (WC) based on the relaxed genome-wide significance threshold $P = 5{\cdot}10^{-6}$.

Method	n_{SNP}	β	95% CI	P-value
Fat intake (E%) → BMI				
IVW (mult. random effects)	20	0.171	(0.040, 0.303)	0.011
Radial MR-Egger	20	0.400	(0.080, 0.880)	0.120
Weighted median	20	0.205	(0.032, 0.378)	0.020
Weighted mode	20	0.232	(−0.006, 0.471)	0.071
Radial IVW (Mod.2nd)	20	0.171	(0.040, 0.303)	0.011
Radial IVW (exact fixed effects)	20	0.178	(0.060, 0.297)	0.003
Radial IVW (exact random effects)	20	0.177	(0.042, 0.313)	0.019
RAPS	20	0.178	(0.056, 0.301)	0.004
PRESSO	20	0.171	(0.040, 0.303)	0.019
Fat intake (E%) → WC[a,b,c]				
IVW (mult. random effects)	20	0.237	(0.067, 0.407)	0.006
Radial MR-Egger	20	0.337	(−0.296, 1.025)	0.239
Weighted median	20	0.220	(0.015, 0.425)	0.036
Weighted mode	20	0.102	(−0.300, 0.503)	0.615
Radial IVW (Mod.2nd)	20	0.236	(0.006, 0.407)	0.006
Radial IVW (exact fixed effects)	20	0.249	(0.112, 0.387)	0.001
Radial IVW (exact random effects)	20	0.245	(0.085, 0.405)	0.007
RAPS	20	0.213	(0.016, 0.410)	0.034
PRESSO	20	0.237	(0.067, 0.407)	0.013
Protein intake (E%) → BMI				
IVW (mult. random effects)	20	0.125	(0.013, 0.236)	0.028
Radial MR-Egger	20	0.142	(−0.276, 0.561)	0.514
Weighted median	20	0.062	(−0.099, 0.223)	0.449
Weighted mode	20	0.000	(−0.291, 0.292)	0.999
Radial IVW (Mod.2nd)	20	0.125	(0.013, 0.237)	0.028
Radial IVW (exact fixed effects)	20	0.129	(0.021, 0.238)	0.020
Radial IVW (exact random effects)	20	0.129	(0.007, 0.251)	0.052
RAPS	20	0.129	(0.017, 0.242)	0.024
PRESSO	20	0.125	(0.013, 0.236)	0.041
Protein intake (E%) → WC				
IVW (mult. random effects)	20	0.169	(0.060, 0.279)	0.002
Radial MR-Egger	20	0.213	(−0.193, 0.619)	0.318
Weighted median	20	0.108	(−0.076, 0.291)	0.224
Weighted mode	20	−0.030	(−0.351, 0.286)	0.842
Radial IVW (Mod.2nd)	20	0.169	(0.060, 0.279)	0.002
Radial IVW (exact fixed effects)	20	0.174	(0.049, 0.298)	0.006
Radial IVW (exact random effects)	20	0.174	(0.055, 0.292)	0.010
RAPS	20	0.174	(0.044, 0.303)	0.008
PRESSO	20	0.169	(0.060, 0.279)	0.007

Abbreviations: IVW (mult. random effects), inverse-variance weighted (multiplicative random effects); Mod.2nd, modified second-order weights; RAPS, Robust Adjusted Profile Score; PRESSO, Pleiotropy RESidual Sum and Outlier.
[a] Possible pleiotropy revealed by MR PRESSO global test.
[b] Possible horizontal pleiotropy revealed by Cochran's Q-statistic.
[c] Suggested MR-Egger over the IVW method by difference of Q−Q′ and ratio Q′/Q.

In a final sensitivity analysis, we identified SNPs that might influence the outcome on a path through a confounder of the exposure-outcome association rather than through the appropriate exposure. Regarding a PhenoScanner search, we excluded all alcohol, smoking, education, as well as physical activity-related SNPs (Supplementary Table 10). After excluding all confounder-related SNPs, 56 instruments remained for BMI, 32 for WC, 21 for fat and protein intake (E%), and 5 for carbohydrate intake (E%) to be used in the sensitivity analysis (Supplementary Tables 4 and 16). Three models (i.e. relative carbohydrate intake on BMI, WC on relative fat intake, and relative protein intake on WC) showed different signs for radial MR-Egger or weighted mode point estimates before and after SNP exclusion. All other approaches provided consistent estimates (Figs. 3–5).

3.2.3. Causal effect estimates from multivariable approach

The multivariable IVW multiplicative random effects models of the genetically predicted relative carbohydrate intake (E%), adjusted for relative proportions of fat and protein, revealed strong inverse effects on both BMI and WC (Fig. 6). The estimates for BMI ($\beta_{IVW} = -0.441$ per 1 SD; 95% CI: −0.772, −0.109; P-value = 0.009) and WC ($\beta_{IVW} = -0.410$ per 1 SD; 95% CI: −0.666, −0.154; P-value = 0.002) were similar to the univariable estimates, indicating robust results. In contrast, no notable effects of relative fat and protein intake (E%) on the anthropometric measures could be observed after mutual adjustment.

The conditional F-statistics for relative carbohydrate intake in the models on BMI ($F = 8.8$) and WC ($F = 8.1$) were below 10, indicating weak instruments. Additionally, heterogeneity of the effect on BMI ($Q = 31.6$; P-value = $5{\cdot}10^{-4}$) but not on WC ($Q = 16.8$; P-value = 0.113) could be observed (Supplementary Table 24). However, the switch to the Q-minimization approach resulted in slightly lower but consistent point estimates $\beta_{IVW} = -0.381$ SD change in BMI and $\beta_{IVW} = -0.325$ SD change in WC per 1 SD change in carbohydrate intake (E%) (Fig. 6). These effects were confirmed by consistent radial MR-Egger and Median estimates within further sensitivity analyses (Supplementary Fig. 2).

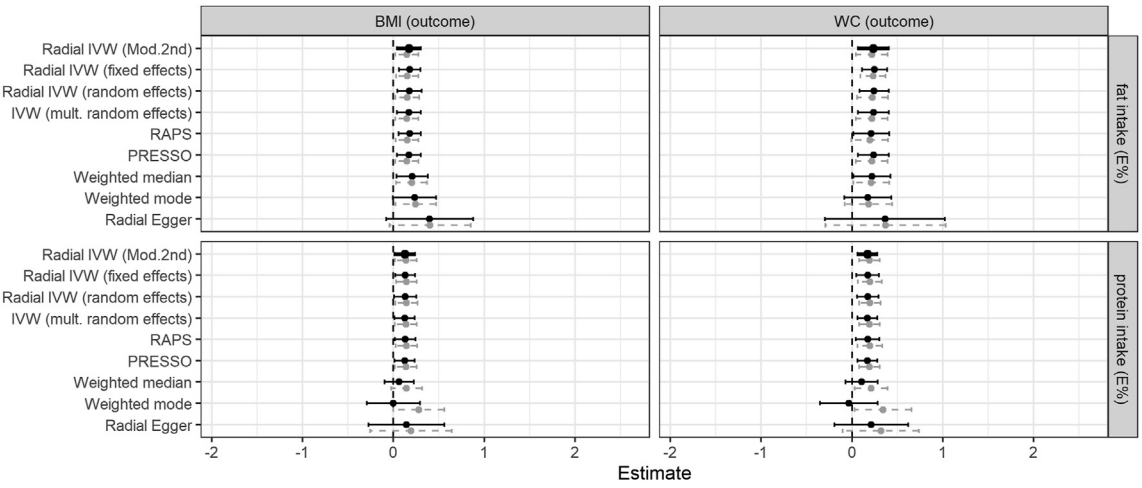

Fig. 5. Sensitivity analysis: Causal estimates and 95% confidence intervals (standard deviation (SD) change in each outcome per one SD change in the respective exposure) of the impact of relative dietary intake of fat and proteins (in % of total energy intake (E%)) on body mass index (BMI) and waist circumference (WC) based on a relaxed genome-wide significance threshold $P = 5 \cdot 10^{-6}$. Bold lines illustrate the main analyses. Grey points with dashed confidence intervals represent effects of sensitivity analyses after exclusion of potential confounder-associated single nucleotide polymorphisms (SNPs) due to control of horizontal pleiotropy. Abbreviations: IVW (mult. random effects), inverse-variance weighted (multiplicative random effects); Mod.2nd, modified second-order weights; RAPS, Robust Adjusted Profile Score; PRESSO, Pleiotropy RESidual Sum and Outlier.

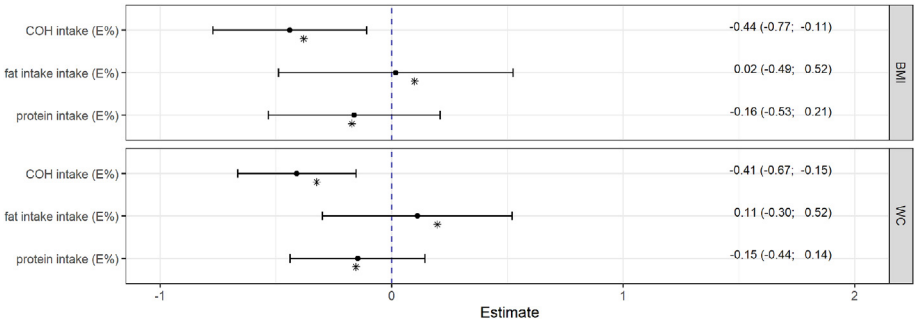

Fig. 6. Mutually-adjusted causal estimates (standard deviation (SD) change in each outcome per one SD change in the respective exposure) from multivariable Mendelian randomization analyses of genetically predicted relative dietary intake of carbohydrates, fat, and proteins (in % of total energy intake (E%)) on the anthropometric measures body mass index (BMI) and waist circumference (WC). Estimates with 95% confidence intervals were obtained from the inverse-variance weighted method with multiplicative random effects. Point estimates shown as asterisks were obtained from the Q-minimization approach that account for both substantial heterogeneity and weak instruments.

4. Discussion

In the present study we conducted MR analyses using large-scale GWAS summary statistics to investigate causal relationships of dietary macronutrient composition (as proportions of total energy intake) with BMI and WC and vice versa. We found that genetically predicted intake of carbohydrates (E%) reduced BMI and WC. Genetic predisposition to relative fat and protein intake was suggestively positively related to both anthropometric measures. There was evidence for bidirectional effects between relative fat intake and both BMI and WC. However, there was no evidence for an effect of both measures on the proportion of carbohydrates in the diet, but on the basis of the analyses carried out, it remains unclear whether this weak causal effect in the reverse direction is reflective of no causal effect or limited power to detect such an effect.

4.1. Carbohydrate intake and anthropometric measurements

Some observational studies reported that a higher proportion of carbohydrates in unrestricted diets were associated with a decrease in WC [17,19,20]. However, an intervention study by Claessen et al. showed an increase in WC with a high-carbohydrate diet [21]. Furthermore, most epidemiological studies reported an inverse association between carbohydrate intake and BMI; these results were underlined by intervention studies reporting weight loss in connection with a high carbohydrate/low fat diet [22]. In this context, the type of carbohydrates certainly plays an essential role. Carbohydrates include subtypes, which may contribute to differing biologic and clinically relevant effects. A diet rich in more complex carbohydrates and dietary fiber may be associated with an overall lower energy intake [17] and an increase in satiety [23]. In particular, the intake of fruits and vegetables increases the consumption of dietary fiber [20]. Unfortunately, in our study the carbohydrate measure does not distinguish between potentially “healthier” (unrefined) and “unhealthier” (refined) carbohydrates. In addition, there is a large body of systematic reviews on the effect of high-carbohydrate/low-fat versus low-carbohydrate/high-fat diets; in summary, there were no noticeable differences between these diets with regard to weight loss. In healthy subjects, the consumption of carbohydrates leads to an acute increase in carbohydrate oxidation and only to a slight increase of de-novo lipogenesis [24,25]. On the contrary, a systematic review and meta-analysis of clinical trials could show that low carbohydrate diets were associated with a decrease in body weight, BMI, abdominal circumference, and other cardiovascular risk factors. However, that meta-analysis was based

on 1141 obese patients and not healthy subjects [26]. Thus, one should be cautious in extrapolating findings from intervention studies on macronutrient intake and weight loss in obese persons to the role of macronutrients in the prevention of weight gain in the general population.

The finding that a proportionally higher content of carbohydrates has an inverse effect on both anthropometric measures may be explained by several related mechanisms. The three macronutrients have a different effect on meal-induced thermogenesis that is the increase of energy expenditure above resting levels for 4–8 h after food ingestion; 20–30% of energy intake from protein, 5–10% from CHO, and 0–3% from fat are spent as meal-induced thermogenesis [27]. A prior clinical trial has shown that the consumption of a carbohydrate meal was associated with a two-fold higher meal-induced thermogenesis in comparison with fat consumption [28], a fact that may contribute to the favorable effect of carbohydrate consumption on obesity measures. Furthermore, many high-carbohydrate foods are low in energy density, which may delay the rate of gastric emptying as well as increase satiety and thereby contribute to a reduction of obesity risk [29,30]. In addition, a prior study found that at higher levels of total energy intake relative protein intake declines, while carbohydrate intake remain constant and fat intake increases [13]. Clinical studies have shown that the body handles fat intake differently than it does with the consumption of carbohydrates. Contrary to carbohydrate intake, which stimulates carbohydrate oxidation [30], the consumption of dietary fat does not cause an increase in fat oxidation [31]; due to the higher energy density of fat and its low satiating effect, fat - contrary to carbohydrate intake - may lead to food and energy overconsumption and an increased obesity risk [32].

As the role of carbohydrate intake on measures of obesity was inconclusive so far, the results of the present study could help to clarify this connection. We found a causal inverse effect of relative carbohydrate intake on BMI and WC., while the reverse direction was less clear due to observed heterogeneity and weaker, but almost unanimously negative effect sizes. Macronutrient intake is a genetically complex phenotype [33,34]. Prior studies have reported that diet composition is associated with other phenotypes and hence it may share genetic components with lifestyle factors, socioeconomic status and health [13,35]. Because it is likely that macronutrient intake is genetically correlated with a number of other factors, the present findings should be carefully interpreted. Nevertheless, the strong effect of carbohydrate intake on BMI and WC warrant further attention.

4.2. Fat and protein intake and anthropometric measurements

Genetically predicted fat and protein intake was suggestively positively associated with both anthropometric measures. However, in the other direction, a distinct positive effect of BMI and WC on fat and protein intake (E%) was found. These bidirectional effects might give rise to a vicious circle. According to Blundell et al. [36], excess fat intake is associated with increased fat deposition. A Cochrane Review including 37 randomized controlled trials (57,079 participants) found that there is consistent high-quality evidence that lower fat intake versus a high fat intake leads to a small but noticeable decrease in body weight, BMI, WC, and percentage of body fat [37]. Dietary fat increases palatability and energy-content of foodstuff while not sufficiently inducing short-term satiety, thus leading to an overall higher calorie intake and energy overconsumption [36]. Fat provides more energy per gram (~9 kcal/g) than carbohydrates or proteins (~4 kcal/g). Therefore, in unrestricted diets, a high dietary fat intake is associated with weight gain [23]. It was also shown that obese subjects tend to consume a diet with a higher fat content than normal weight subjects [38], which underlines our results that a higher BMI or WC increases higher fat consumption. We could only investigate the impact of total fat intake (E%) on the outcomes; because there is evidence that specific types of dietary fat could exert differential effects on energy metabolism and storage [39], further studies are necessary to focus on the effect of fat quality on BMI and WC.

In contrast to the changing patterns of carbohydrate and fat consumption, the intake of protein remained relatively stable across international populations and US demographic groups over the last decades [40]. Regarding weight loss, diets rich in protein were reported to be effective due to their impact on appetite, satiety, and eating-related thermogenesis [41,42]. However, a meta-analysis including 15 randomized controlled trials (duration $\geq$ 12 months) found no effects of high-protein intake versus normal intake on body weight and WC [43]. Relatively few studies focused on the role of habitual protein intake on body composition [44–46]; unexpectedly, high habitual protein intake (E%) contributed to body weight, BMI, WC, and percentage body fat [47] in the general population [44,46–48]. In a prospective study including 22,000 participants, high total and animal protein intake was related to an increase in BMI over time [49]. In the present study, no differentiation between animal-derived and plant-derived protein intake was possible; most likely animal-derived protein plays a more important role regarding weight gain than plant-based proteins [50,51]. Further studies are necessary to determine the mechanisms underlying the effect between protein intake and BMI as well as WC and vice versa.

4.3. Strengths and limitations

The major strength of this study is that we - for the first time - assessed the causal effects of relative carbohydrate, fat, and protein intake on BMI and WC using a bidirectional MR approach. The MR analysis is less susceptible to problems of confounding, reverse causation and exposures non-differentially measured with error in comparison to conventional observational studies [52]. Furthermore, in addition to the IVW method, we conducted a number of sensitivity analyses to ensure the consistency of causal estimates and confirm robustness of the present findings. We also performed a multivariable analysis that strengthened our findings of the impact of relative carbohydrate intake on both anthropometric measures. In addition, by using two-sample summary statistics data from GWAS including large sample sizes, our study had high statistical power to estimate reliable causal effects, while small sample overlaps ensured almost unbiased estimates.

The present study also has limitations. The genetic instruments for macronutrient intake explained only a small fraction of phenotypic variability. Furthermore, there were too few genome-wide significant SNPs with the relative proportions of fat and protein intake, which led to convergence problems of optimization algorithms within MR methods, disproportionately high SNP-specific impacts accompanying by large Q-statistics, abnormally large overdispersion and therefore (if calculation was possible) to unreliable estimates. Thus, we relaxed the P-value threshold for instrument selection to increase the number of SNPs, which may be accompanied by higher pleiotropy and weak instrument bias. By assessing a variety of sensitivity methods including the MR-RAPS approach, we tried to eliminate this shortcoming. We also considered SNP-specific F-statistics, to assess the predisposition to bias due to weak instruments. Another limitation is that MR-Egger tests and estimates that are accompanied by low power and susceptibility to regression dilution bias. In the present study, causal estimates in the analyses were obtained for macronutrient intakes proportional to total energy intake. This standard procedure in nutritional epidemiology allows focusing on effects of the specific

macronutrients independent of total energy intake. It also attenuates effects of misreporting in dietary assessment. Thus the analysis of energy-adjusted macronutrient intakes (i.e., expressed as % of total energy intake) is clearly superior to an analysis of macronutrient intakes in grams per day; accordingly, such analyses were not conducted by Meddens et al. [13].

Furthermore, based on given summary level data we could investigate neither gender-specific differences nor effects between macronutrient subtypes. The data do not distinguish between "healthier" and "more unhealthy" carbohydrates, so in the present study only statements for the total amount of carbohydrates can be made regardless of the carbohydrate quality. In the SSGAC data, information on the relative total sugar intake is available (as a subgroup of relative carbohydrate intake). Information on the quality of dietary carbohydrates, fat, and protein is lacking, except for sugar intake data. Thus, we had to focus on the effects of the total intake of carbohydrates, fat and protein in our work. Finally, the present findings were based on individuals of European descendent and may not be generalizable to other ethnicities.

5. Conclusions

In conclusion, we could show that a higher proportion of carbohydrates (E%) in the diet reduces BMI and WC. There were bidirectional effects between relative dietary intake of fat and anthropometric measures, which might give rise to a vicious circle. These results support findings from observational studies that higher relative fat intake at the expense of carbohydrate intake increases BMI and WC, eventually leading to overall and abdominal obesity. Further research focusing on the quality of dietary carbohydrate, fat, and protein intake and possible consequences on micronutrient intake is needed to define the implications of our findings for dietary intake recommendations.

Ethics approval

Not applicable, since the study based on summary-level data. In all original studies, ethical approval had been obtained.

Data availability

The present study based on summary-level data that have been made publicly available. Summary data from genome-wide association studies for BMI (Locke et al.) and WC (Shungin et al.) are available at https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files. The GWAS of relative macronutrients-intake are available at https://www.thessgac.org/data.

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Author contributions

DF analyzed the data and created the tables and plots. CM did the literature research and contributed together with DF to the data collection. All authors wrote the manuscript and contributed to data interpretation. JL and CM finally reviewed the manuscript. All authors read and approved the final version.

Conflict of interest

The authors declare that they have no competing interests.

Abbreviations

BMI	Body Mass Index
WC	Waist Circumference
CI	Confidence Interval
COH	Carbohydrate
E%	% of total Energy intake
GWAS	Genome Wide Association Study
MR	Mendelian Randomization
SNP	Single Nucleotide Polymorphism
IVW	Inverse-Variance Weighted
LD	Linkage Disequilibrium
RAPS	Robust Adjusted Profile Score
PRESSO	Pleiotropy RESidual Sum and Outlier
SD	Standard Deviation
SE	Standard Error
InSIDE	Instrument Strength Independent of Direct Effect

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.01.047.

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