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Evaluating causal associations between chronotype and fatty acids and between fatty acids and type 2 diabetes: A Mendelian randomization study

Charleen D. Adams*, Susan L. Neuhausen

Department of Population Sciences, Beckman Research Institute of City of Hope, Duarte, CA, USA

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Abstract *Background and aims:* Preference for activity in the morning or evening (chronotype) may impact type 2 diabetes (T2D) risk factors. Our objective was to use Mendelian randomization (MR) to evaluate whether there are causal links between chronotype and one potential T2D risk factor, total fatty acids (TOTFA), and between TOTFA and T2D.
Methods and results: We estimated the causal effect of: 1) morning chronotype on TOTFA; and 2) higher TOTFA on T2D. We found that: a) morning compared to evening chronotype was associated with lower TOTFA levels (inverse-weighted variance (IVW) estimate −0.21; 95% CI −0.38, −0.03; raw $P = 0.02$; FDR-corrected P 0.04) and b) elevated TOTFA levels were protective against T2D (IVW estimate −0.23; 95% CI −0.41, −0.05; raw $P = 0.01$; FDR-corrected $P = 0.03$). Based on this finding, we further hypothesized that healthy fats would show a similar pattern and performed MR of a) morning chronotype on omega-3 (Omega-3), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids; and b) MR of each of these fat types on T2D. We observed the same mediating-type pattern for chronotype, MUFA, and T2D as we had for chronotype, TOTFA, and T2D, and morning chronotype was associated with lower Omega-3.
Conclusion: Our findings provide suggestive, new information about relationships among chronotype, TOTFA, and T2D and about chronotype as a factor influencing Omega-3, MUFA, and TOTFA levels. In addition, we validated previous knowledge about MUFA and T2D. Morning chronotypes may predispose towards lower levels of TOTFA and some healthy fats, whereas higher levels of TOTFA and MUFA may protect against T2D.

Introduction

Chronotype, a preference for activity in the morning or evening, has a strong genetic component, including, but not limited to, genes that are part of the circadian clock [1]. Circadian-clock genes form a transcription-translation feedback loop that produces daily rhythms in many physiologic parameters, including sleep–wake activity, hormone production, and metabolism [2]. In a homozygous *Clock*-mutant mouse, the mutation caused obesity and metabolic syndrome [3]; *Bmal1*-deleted mice had elevated levels of some circulating fatty acids [4] and developed diabetes secondary to misregulation of mitochondrial energy metabolism and impaired glucose-stimulated insulin secretion [5]. In humans, chronotype has been associated with the timing of intake of dietary fats [6]. Circulating fatty acids may be a source of inflammation that underlies insulin-resistance, a feature of type

* Corresponding author.
E-mail address: chaaadams@coh.org (C.D. Adams).

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2 diabetes (T2D) [7]; however, a recent meta-analysis of the intake of total fatty acids (TOTFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) did not find an association between these fats (unlike with trans fatty acids) and cardiovascular disease, a frequent comorbidity of T2D [8]. Chronotype may impact adaptability to changes in diet, work, and sleep schedules; these changes can lead to circadian disruption – a misalignment of behavior and biological rhythms [9]. Observational studies suggest it may play a role in developing T2D [10–12].

Though validated questionnaires are often used, the chronotype phenotype can be assigned by asking people whether they prefer to be awake in the early mornings (self-reported morning chronotype) or late evenings (self-reported evening chronotype). This self-reported value was used in the Mendelian randomization (MR) analysis by Jones et al. (2018) of the causal (versus observational) effect of morning chronotype on T2D. MR is an instrumental-variables technique that uses germline genetic variants as instruments – "proxies" for independent variables to avert confounding and reverse causation to which observational designs are prone. In contrast to the observational studies referenced above, Jones et al. (2018) did not find evidence that chronotype causes T2D [13]. One explanation for the discrepancy between the observational studies suggesting that chronotype impacts metabolic outcomes and the Jones' MR that found no causal association is that there may be different effects of chronotype on T2D risk factors, and these effects may cancel each other out. This may have led to the null association found by the Jones' MR study. It is possible that chronotype causally affects important individual T2D risk factors. Finding out which T2D risk factors chronotype impacts and the direction of the effects could provide potentially useful information as to how best to modify behavior in order to prevent metabolic disorders. For instance, evening chronotype has been associated with sedentary behavior and other lifestyle factors that may explain the risk of evening chronotype on metabolic syndrome; however, evening chronotype was not causally associated with metabolic syndrome in an MR study [14]. Thus, teasing out how chronotype impacts metabolic risk factors is a challenge. It is unclear whether one potential risk factor, fatty acids, promote or protect against development of T2D. Our objective was to examine the causal impact of chronotype on fatty acids, and to examine the causal impact of this T2D risk factor on T2D. To get a bird's eye view, we focused on total fatty acids (TOTFA). We used MR to avoid confounding and reverse causation.

Methods

Conceptual approach

We performed MR with the assumptions that the instrumental variables are: 1) strongly associated with the exposures; 2) not associated with any confounders of the exposures and outcomes; and 3) only associated with the outcomes through the exposures.

Single nucleotide polymorphisms (SNPs) are unlikely to be associated with confounders of exposure-outcome relationships due to the essentially random assortment of alleles at gamete production and fertilization, making them attractive as instruments in MR. We used SNPs strongly associated with the exposures ($P < 5e{-}08$) as our instruments. We combined harmonized SNP-exposure and SNP-outcome associations into multi-allelic models, using the inverse-variance weighted (IVW) method [15], which treats each SNP in the model as a natural experiment and provides a meta-analysis of the Wald ratios [16,17].

For all relationships and replications, where the exposure SNPs were not available in the outcome data, we identified proxy SNPs in linkage disequilibrium ($R2 > 0.8$ and within 250 kb of the target SNP). In order to assess weak-instrument bias, which can reduce statistical power when an instrument explains a limited proportion of the variance in an exposure, we calculated estimates of the proportion of variance in each exposure explained by the genetic instruments (R^2) and the strength of the association between the genetic instruments and the exposure (*F*-statistics); $F > 10$ is used as a guide for judging that a genetic instrument is less likely to suffer from weak-instrument bias, which can inflate the possibility of false positives [18]. We performed all MR analyses using the "TwoSampleMR" package [16] in R version 3.5.1. All data sources used are publicly available through MR-Base (and accessible through the "TwoSampleMR" package), a curated database of complete GWASs and platform for performing 2-sample MR [16].

Main MR analysis: chronotype, TOTFA, and diabetes/T2D

We examined the causality of the following four relationships (Fig. 1):

1. Self-reported morning chronotype (m-chronotype) on circulating total fatty acids (TOTFA)
2. TOTFA on m-chronotype
3. TOTFA on self-reported clinical diagnosis of diabetes (diabetes)
4. Diabetes on TOTFA

Relationships 1 and 2 are a bidirectional analysis of m-chronotype and TOTFA. Relationships 3 and 4 are a bidirectional analysis of TOTFA and diabetes. Bidirectional analyses help determine the direction of causality for an association. Because the validity of bidirectional MR methods depends on the independence of the two instruments [19], we checked that there was no overlap or linkage disequilibrium (LD) between the genetic variants included in the instruments for m-chronotype and TOTFA and the instruments for TOTFA and diabetes. We used LDassoc, a free and publicly available web tool, to assess whether instruments were in LD [20].

Relationships 1 and 3 comprise a 2-step MR, which is similar conceptually to mediation, a statistical approach to

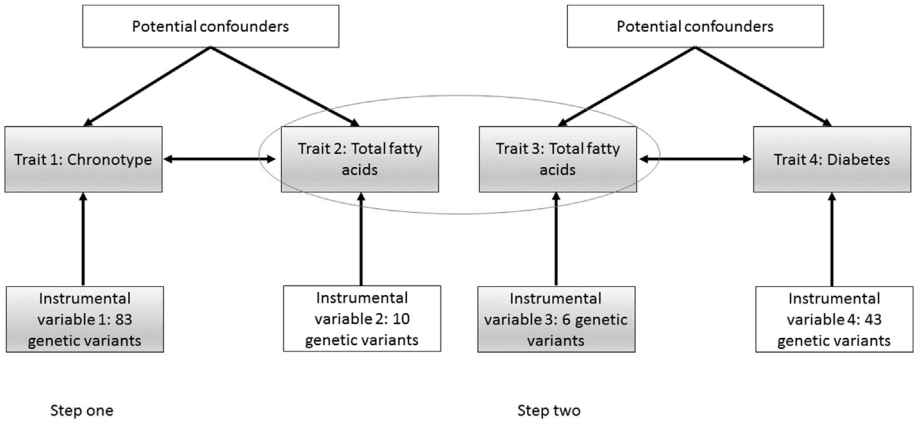

Figure 1 Diagram showing the MR analyses of chronotype, total fatty acids, and diabetes with 2-step and bidirectional approaches.

study a proposed mechanism: whether some trait affects another through the presence of a third trait (the mediator). In this case, if the proxy for m-chronotype influences TOTFA and the proxy for TOTFA influences diabetes, then this suggests an indirect influence of m-chronotype on diabetes through TOTFA.

Sources of genome-wide association study (GWAS) summary statistics data

UK Biobank (UKBB)

Details about the UKBB population have been described elsewhere [21]. In brief, to obtain summary data for chronotype, we obtained data from 301,143 unrelated individuals of British ancestry, for which UKBB had collected a single self-reported measure of chronotype (respondents were asked whether they were morning or evening chronotypes). The GWAS of chronotype (binary variable with morning type scored as 1 and evening type as 0) was run by the Neale lab, which adjusted the analysis for sex and 10 principal components and made their results publicly available [22]. Similarly, to obtain summary statistics for diabetes, we used a GWAS of diabetes run by the Neale lab, which also used UKBB data. For this, *diabetes* was defined as "diabetes diagnosed by a physician". As above, this GWAS was adjusted for sex and 10 principal components. There were 336,473 individuals in the diabetes analysis.

Population-based European Cohort (PBEC)

We obtained summary statistics for fatty acids from Kettunen et al. (2016), who performed a meta-analysis of 14 European cohort GWASs for circulating metabolic traits, including TOTFA (n = 13,505) and other fatty acids. The details of the cohorts included in PBCE have been described elsewhere [23]; briefly, the population was an average age of 46 years, an average BMI of 26, and 54% female. PBEC adjusted its metabolic data for age, sex, time from last meal, and where possible, the first 10 principal components from the genomic data. The metabolic traits were measured by a quantitative high-throughput nuclear magnetic resonance (NMR) metabolomics platform and transformed into standard deviation units.

DIAbetes Genetics Replication and Meta-analysis (DIAGRAM)

Because the UKBB's question of whether a patient was ever diagnosed with diabetes potentially included some type 1 diabetes cases, we chose to replicate relationships 3 and 4 with a smaller T2D-specific GWAS. We obtained summary statistics for T2D from DIAGRAM, which performed a GWAS of T2D in 149,821 individuals and adjusted its GWAS data for study-specific covariates and population structure [24].

Sensitivity analyses

For each relationship, we performed sensitivity analyses using IVW radial regression to detect presence of horizontal pleiotropy, which would violate the instrumental variable assumptions of MR. IVW radial regression uses the *P*-value from Cochran's *Q*-statistic (*P*[het]) to indicate possible heterogeneity among the instruments' ratio estimates [25]; pleiotropy is one reason estimates might be heterogenous. When heterogeneity was detected, we removed outliers and repeated both the analysis and the IVW radial regression. Instruments with no evidence for heterogeneity were selected for the final models, except when removing outliers would have made an instrument have fewer than five SNPs. For these models with evidence of heterogeneity and no more than five SNPs, we applied MR-PRESSO, which detects and corrects for pleiotropic outliers in multi-instrument MR settings [26].

We also performed MR-Egger regression. MR-Egger is the adaption of a tool to detect small-study bias in meta-analysis [15] that can provide unbiased estimates when all SNPs in an instrument are invalid due to horizontal pleiotropy. The direction and strength of the effect of the MR-Egger estimate can be used to partially triangulate the IVW estimate findings, even if the MR-Egger estimate lacks precision. In addition to MR-Egger, we computed weighted median and weighted mode estimators, as these make

different assumptions about the potential nature of underlying pleiotropy. When IVW, MR-Egger, weighted median, and weighted mode estimators are comparable, it strengthens the confidence we can have in the validity of the instruments [27].

M-Chronotype on TOTFA: Relationship 1

We constructed a genetic instrument using 83 m-chronotype-associated SNPs from the Neale lab's GWAS of chronotype by extracting their effect sizes and standard errors (summary statistics) and obtained the corresponding SNP—outcome (i.e., TOTFA) association by extracting the summary statistics of the m-chronotype-associated SNPs from PBEC.

TOTFA on m-chronotype: Relationship 2

For TOTFA, we constructed a genetic instrument using 10 of 12 available TOTFA-associated SNPs in PBEC (2 removed after sensitivity analysis for heterogeneity) [23] and obtained the SNP-outcome association (i.e., m-chronotype) summary statistics from the Neale lab's GWAS of m-chronotype [22].

TOTFA on diabetes: Relationship 3

For TOTFA, we constructed a genetic instrument using 6 of 12 available TOTFA-associated SNPs (6 removed after sensitivity analysis for heterogeneity), as estimated in PBEC [23] and obtained the corresponding SNP—outcome (i.e., diabetes) summary statistics from the Neale lab's GWAS of diabetes [22].

Diabetes on TOTFA: Relationship 4

For diabetes, we constructed a genetic instrument using 43 of 47 (4 removed after sensitivity analysis for heterogeneity) diabetes-associated SNPs, as estimated from the Neale lab's GWAS of diabetes [22] and obtained the corresponding SNP-outcome association (i.e., for the TOTFA outcome) summary statistics from PBEC.

TOTFA on T2D and T2D on TOTFA: Replication of relationships 3 and 4

We constructed a genetic instrument using five TOTFA-associated SNPs from PBEC and took the SNP-outcome association from DIAGRAM. For the reverse, we constructed a genetic instrument using 33 T2D-associated SNPs from DIAGRAM (3 SNPs were removed in the sensitivity analysis for heterogeneity) and obtained the corresponding SNP-outcome summary statistics from PBEC.

M-chronotype, Omega-3, and T2D

Step 1 — m-chronotype on Omega-3

We constructed a genetic instrument using 83 m-chronotype-associated SNPs from the Neale lab's GWAS of m-chronotype and obtained the SNP—outcome (i.e., Omega-3) summary statistics from PBEC [23].

Step 2 — Omega-3 on T2D

For Omega-3, we constructed a genetic instrument using 5 Omega-3-associated SNPs, as estimated in PBEC [23] and the corresponding SNP—outcome (i.e., T2D) summary statistics from DIAGRAM [24].

M-chronotype, MUFA, and T2D

Step 1 — m-chronotype on MUFA

For m-chronotype, a genetic instrument was constructed using 78 of 83 m-chronotype-associated SNPs (5 removed after sensitivity analysis for heterogeneity), as estimated in UKBB [21] and the corresponding SNP—outcome (i.e., MUFA) summary statistics from PBEC [23].

Step 2 — MUFA on T2D

For MUFA, a genetic instrument was constructed using 3 MUFA-associated SNPs, as estimated in PBEC [23]. To generate the corresponding SNP—outcome (i.e., T2D) association, we took summary statistics from DIAGRAM [24].

M-chronotype, PUFA, and T2D

Step 1 — m-chronotype on PUFA

For m-chronotype, a genetic instrument was constructed using 83 m-chronotype-associated SNPs. To generate the corresponding SNP—outcome (i.e., PUFA) association, we took summary statistics from PBEC [23].

Step 2 — PUFA on T2D

For PUFA, a genetic instrument was constructed using 5 PUFA-associated SNPs, as estimated in PBEC [23]. To generate the corresponding SNP—outcome (i.e., T2D) association, we took effect summary statistics (DIAGRAM) [24].

Multiple testing

We performed 12 tests and applied the Benjamini-Hochberg false-discovery rate (FDR) procedure to adjust the raw *P* values for multiple testing [28].

Results

M-chronotype on TOTFA: Relationship 1

We observed a decrease of 0.21 (95% CI −0.38, −0.03; $P = 0.02$; FDR-corrected P 0.04) standard deviation (SD) units of TOTFA for morning compared to evening chronotype (total R^2 for instrument = 27.12%; *F*-statistic = 60.27) (Table 1). IVW radial regression sensitivity analysis indicated no evidence for heterogeneity (P [het] = 0.09). The association was robust to MR Egger, weighted median, and weighted mode estimates (the betas had the same direction of effect and were of comparable magnitudes (Supplemental Tables 1 and 2 contain

the results for individual SNPs and for radial regression sensitivity analyses, respectively).

TOTFA on m-chronotype: Relationship 2

Although the IVW radial regression indicated no evidence of heterogeneity (P[het] = 0.70) and the multiple sensitivity estimators were comparable, there was a null effect of TOTFA on m-chronotype, with a 0.002 (95% CI −0.01, 0.02; P = 0.79; FDR-corrected P = 0.86) decrease in chronotype (morning to evening) per SD-unit increase in TOTFA (total R^2 for instrument = 4.24%; F-statistic = 59.81) (Table 1; Supplemental Tables 3–6 contain results for individual SNPs and for the radial regression sensitivity analysis before and after removal of outliers).

TOTFA on diabetes: Relationship 3

We observed no evidence for heterogeneity in the IVW radial regression (P[het] = 0.73). There was a 0.006 (95% CI −0.011, −0.002; P = 0.009; FDR-corrected P = 0.03) decrease in diabetes per SD increase in TOTFA that was robust across estimators (total R^2 for instrument = 2.45%; F-statistic = 56.57) (Table 1; Supplemental Tables 7–10 contain results for individual SNPs and for the radial regression sensitivity analysis before and after removal of outliers).

TOTFA on T2D: Replication of relationship 3

The causal effect of TOTFA on diabetes replicated in the DIAGRAM cohort (IVW estimate −0.23; 95% CI −0.41, −0.05; P = 0.01; FDR-corrected P = 0.03) total R^2 for instrument = 3.26%; F-statistic = 90.89) and was robust across estimators, though IVW radial regression detected some evidence of heterogeneity (P[het] = 0.04). We used MR-PRESSO to account for heterogeneity (MR-PRESSO beta −0.13; 95% CI −0.20, −0.05; P = 0.04) (Table 1; Supplemental Tables 11 and 12 contain results for individual SNPs and for the radial regression identifying outliers).

Diabetes on TOTFA: Relationship 4

We observed a 0.87 (95% CI −0.02, 1.77; (P = 0.06; FDR-corrected P = 0.10) null increase in TOTFA for those diagnosed with diabetes compared to those without diabetes (total R^2 for instrument = 20.91%; F-statistic = 82.73). While there was no evidence for heterogeneity (P[het] = 0.78), there were inconsistencies between the sensitivity estimators, indicating potential pleiotropy (Table 1; Supplemental Tables 13–16 contain results for individual SNPs and for the radial regression sensitivity analysis before and after removal of outliers).

T2D on TOTFA: Replication of relationship 4

We observed no evidence for heterogeneity (P[het] = 0.88). There was a 0.03 null increase in TOTFA for those with T2D compared to those without it (95% CI −0.01, 0.08; P = 0.15; FDR-corrected P = 0.20; effect-allele frequencies not available for calculation of R^2 and F-statistic), which was robust across the sensitivity estimators (Table 1; Supplemental Tables 16–20 contain the results for IVW of individual SNPs and for the radial regression sensitivity analysis before and after removal of outliers).

Follow-up MR results of healthy fats

Because we observed a protective effect of TOTFA on diabetes/T2D (see above), we tested whether m-chronotype impacts fats the American Diabetes Association (ADA) has defined as healthy (Omega-3, MUFA, and PUFA) and whether these three healthy fats have protective effects on T2D.

M-chronotype, Omega-3, and T2D

Step 1 – m-chronotype on Omega-3

We observed no evidence for heterogeneity (P[het] = 0.52). There was a decrease of 0.19 (95% CI −0.35, −0.03; P = 0.02; FDR-corrected P = 0.04) standard deviation (SD) units of Omega-3 for morning compared to evening chronotype, which was consistent across sensitivity estimators (total R^2 for instrument = 27.44%; F-statistic = 60.72) (Table 1; Supplemental Tables 21–22 contain results for individual SNPs and for the radial regression sensitivity analysis).

Step 2 – Omega-3 and T2D

We observed a 0.17 (95% CI −0.53, 0.18; P = 0.34; FDR-corrected P = 0.40) null decrease in T2D per SD increase in Omega-3 (total R^2 for instrument = 2.55%; F-statistic = 70.73), inconsistency across the MR estimators, and potential heterogeneity (P[het] = 3.14E-08). Although we used MR-PRESSO to account for this heterogeneity, the finding remained null (MR-PRESSO beta = −0.16; 95% CI −0.34, 0.02; P = 0.32) (Table 1; Supplemental Tables 23 and 24 contain the results for individual SNPs and for the radial regression sensitivity analysis).

M-chronotype, MUFA, and T2D

Step 1 – m-chronotype and MUFA

We observed a decrease of 0.29 (95% CI −0.46, −0.12; P = 0.0008; FDR-corrected P = 0.01) SD units of MUFA for morning compared to evening chronotype, which was robust across MR sensitivity estimators and for which there was no evidence of heterogeneity (P[het] = 0.73) (total R^2 for instrument = 25.10%; F-statistic = 57.81) (Table 1; Supplemental Tables 25–28 contain results for individual SNPs and for the radial regression sensitivity analysis before and after removal of outliers).

Step 2 – MUFA and T2D

We observed consistency across the MR estimators. There was a 0.32 (95% CI −0.56, −0.09; P = 0.006; FDR-corrected P = 0.03) decrease in T2D per SD increase in MUFA (total R^2 for instrument = 1.61%; F-statistic = 74.17) (Table 1; Supplemental Table 29 contains results for individual SNPs; we could not apply either IVW radial

Table 1 Results of the Mendelian randomization analyses.

Analysis	Method	N			Lower	Upper	Raw
		SNPs	Beta	SE	CI	CI	*P*-value
Chronotype[a] on TOTFA[b]	Inverse variance weighted	83	−0.210	0.089	−0.385	−0.035	0.0189
	MR Egger regression	83	−0.153	0.215	−0.575	0.268	0.4775
	MR Egger intercept	83	−0.001	0.004	−0.010	0.007	0.7737
	Weighted median	83	−0.192	0.127	−0.441	0.058	0.1328
	Weighted mode	83	−0.467	0.231	−0.919	−0.015	0.0462
TOTFA on chronotype	Inverse variance weighted	10	0.002	0.008	−0.014	0.019	0.7891
	MR Egger regression	10	0.063	0.037	−0.010	0.135	0.1272
	MR Egger intercept	10	−0.007	0.004	−0.015	0.001	0.1304
	Weighted median	10	0.002	0.011	−0.019	0.023	0.8674
	Weighted mode	10	0.009	0.015	−0.020	0.038	0.5626
TOTFA on diabetes[c]	Inverse variance weighted	6	−0.006	0.002	−0.011	−0.002	0.0094
	MR Egger regression	6	−0.009	0.010	−0.029	0.010	0.4060
	MR Egger intercept	6	0.000	0.001	−0.002	0.002	0.7842
	Weighted median	6	−0.006	0.003	−0.012	0.000	0.0675
	Weighted mode	6	−0.006	0.004	−0.013	0.002	0.2165
Diabetes on TOTFA	Inverse variance weighted	43	0.874	0.457	−0.021	1.769	0.0555
	MR Egger regression	43	−0.279	1.130	−2.494	1.936	0.8060
	MR Egger intercept	43	0.006	0.006	−0.005	0.017	0.2710
	Weighted median	43	0.154	0.680	−1.179	1.488	0.8208
	Weighted mode	43	−0.044	0.842	−1.695	1.607	0.9589
TOTFA on T2D[d]	Inverse variance weighted	5	−0.233	0.092	−0.413	−0.054	0.0109
	MR Egger regression	5	−0.027	0.450	−0.909	0.855	0.9555
	MR Egger intercept	5	−0.025	0.052	−0.127	0.078	0.6704
	Weighted median	5	−0.153	0.077	−0.305	−0.002	0.0474
	Weighted mode	5	−0.127	0.093	−0.310	0.056	0.2455
T2D on TOTFA	Inverse variance weighted	33	0.032	0.022	−0.011	0.075	0.1478
	MR Egger regression	33	−0.016	0.055	−0.124	0.092	0.7735
	MR Egger intercept	33	0.006	0.006	−0.006	0.018	0.3516
	Weighted median	33	0.009	0.033	−0.055	0.073	0.7780
	Weighted mode	33	0.001	0.037	−0.072	0.073	0.9816
Chonotype on Omega-3[e]	Inverse variance weighted	83	−0.187	0.082	−0.348	−0.027	0.0222
	MR Egger regression	83	−0.155	0.200	−0.547	0.237	0.4402
	MR Egger intercept	83	−0.001	0.004	−0.009	0.007	0.8619
	Weighted median	83	−0.075	0.133	−0.335	0.185	0.5714
	Weighted mode	83	−0.121	0.212	−0.536	0.294	0.5693
Omega-3 on T2D	Inverse variance weighted	5	−0.174	0.181	−0.528	0.180	0.3366
	MR Egger regression	5	0.276	0.753	−1.199	1.751	0.7384
	MR Egger intercept	5	−0.056	0.090	−0.232	0.120	0.5801
	Weighted median	5	−0.011	0.104	−0.214	0.193	0.9176
	Weighted mode	5	0.099	0.080	−0.058	0.257	0.2828
Chronotype on MUFA[f]	Inverse variance weighted	78	−0.293	0.087	−0.464	−0.122	0.0008
	MR Egger regression	78	−0.552	0.261	−1.063	−0.041	0.0377
	MR Egger intercept	78	0.005	0.005	−0.005	0.015	0.2954
	Weighted median	78	−0.310	0.129	−0.563	−0.057	0.0163
	Weighted mode	78	−0.770	0.304	−1.365	−0.175	0.0132
MUFA on T2D	Inverse variance weighted	3	−0.324	0.119	−0.558	−0.091	0.0064
	MR Egger regression	3	−0.444	1.235	−2.864	1.977	0.7804
	MR Egger intercept	3	0.014	0.143	−0.266	0.294	0.9382
	Weighted median	3	−0.278	0.100	−0.473	−0.082	0.0054
	Weighted mode	3	−0.167	0.183	−0.527	0.192	0.4575
Chronotype on PUFA[g]	Inverse variance weighted	83	−0.128	0.082	−0.288	0.032	0.1168
	MR Egger regression	83	−0.235	0.196	−0.619	0.150	0.2353
	MR Egger intercept	83	0.002	0.004	−0.005	0.010	0.5521
	Weighted median	83	−0.198	0.129	−0.451	0.055	0.1245
	Weighted mode	83	−0.365	0.213	−0.783	0.053	0.0906
PUFA on T2D	Inverse variance weighted	5	−0.011	0.101	−0.209	0.187	0.9140
	MR Egger regression	5	0.205	0.131	−0.052	0.462	0.2159
	MR Egger intercept	5	−0.062	0.031	−0.123	−0.002	0.1365
	Weighted median	5	0.044	0.031	−0.017	0.104	0.1557
	Weighted mode	5	0.049	0.029	−0.008	0.107	0.1675

[a] Chronotype = self-reported morning chronotype.
[b] TOTFA = circulating total fatty acids.
[c] Diagnosis of diabetes by physician, diabetes.
[d] T2D = type 2 diabetes.
[e] Omega-3 = circulating Omega-3 fatty acids.
[f] MUFA = circulating monounsaturated fatty acids.
[g] PUFA = circulating polyunsaturated fatty acids.

regression or MR-PRESSO with only 3 SNPs in the instrument).

M-chronotype, PUFA, and T2D

Step 1 – m-chronotype and PUFA

Although there was no evidence for heterogeneity (P [het] = 0.44) and consistency across the MR sensitivity estimates, we observed a null decrease of 0.13 (95% CI −0.29, 0.32.; P = 0.12; FDR-corrected P = 0.18) SD units of PUFA for morning compared to evening chronotype (total R^2 for instrument = 27.30%; F-statistic = 63.37) (Table 1; Supplemental Tables 30 and 31 contain results for individual SNPs and for the radial regression sensitivity analysis).

Step 2 – PUFA and T2D

We observed a 0.01 (95% CI −0.21, 0.18; P = 0.91; FDR-corrected P = 0.91) null decrease in diabetes per SD increase in PUFA (total R^2 for instrument = 9.15%; F-statistic = 272.85), evidence for heterogeneity (P [het] = 1.7E-10), and inconsistency in the MR sensitivity estimators. We used MR-PRESSO to account for heterogeneity (MR-PRESSO beta = 0.04; 95% CI −0.04, 0.13; P = 0.41), though the inference remained unchanged (Table 1; Supplemental Tables 32 and 33 contain results for individual SNPs and the radial regression sensitivity analysis).

Discussion

The first step in the 2-step MR of m-chronotype and TOTFA and TOTFA and diabetes estimated that m-chronotype causally lowered TOTFA (P = 0.02; FDR-corrected P = 0.04), but the bidirectional analysis did not find the reverse: TOTFA did not influence m-chronotype (P = 0.79; FDR-corrected P = 0.86). The second step found evidence of a causal, protective effect of elevated TOTFA on diabetes (P = 0.009; FDR-corrected P = 0.03), which was replicated in the DIAGRAM cohort (P = 0.01; FDR-corrected P = 0.03). There was no evidence for a reverse effect (diabetes on TOTFA; P = 0.06; FDR-corrected P = 0.10). In total, these findings support a causal effect of morning compared to evening chronotype on lower TOTFA and a protective effect of elevated TOTFA on T2D.

The protective effect of TOTFA on diabetes/T2D was surprising. This led us to hypothesize that healthy fats might show a similar pattern. We chose to examine fats that the ADA refer to as healthy (Omega-3, MUFA, and PUFA). We found that m-chronotype causally lowered MUFA (P = 0.0008; FDR-corrected P = 0.01), lowered Omega-3 (P = 0.02; FDR-corrected P = 0.04), and did not alter PUFA (P = 0.12; FDR-corrected P = 0.18); higher MUFA was causally protective against T2D (P = 0.006; FDR-corrected P = 0.03). No clear causal effects were observed for Omega-3 and PUFA on T2D (P = 0.34 and FDR-corrected P = 0.40; P = 0.91 and FDR-corrected P = 0.91, respectively).

Because the UKBB coded its GWAS such that morning chronotype was the at-risk group, our examination of chronotype is investigating risk for morning chronotype, but our findings are relevant for both chronotypes. If we reverse the signs so that evening chronotype is the risk group, a clearer picture emerges: evening chronotype is linked to higher TOTFA. Similarly, evening chronotype is linked to increases in levels of Omega-3 and MUFA. This suggests that there may be something inherent in evening chronotype that leads to higher levels TOTFA which include Omega-3 and MUFA. Determining why this is may lead to ideas for interventions to increase these circulating fatty acids.

There were two strengths of this MR investigation. First, it enabled an assessment of the direction of causality between m-chronotype and TOTFA and TOTFA and diabetes/T2D; this is an improvement on previous observational designs that were unable to distinguish the direction of effect due to possible reverse causation. Second, we used publicly available data from large-scale GWASs to capitalize on the power of large samples. Further to this, our use of large datasets in a 2-sample approach reduced the probability of Type 1 errors from weak-instrument bias (where instrumental SNPs explain a small proportion of the variance in an exposure) [29].

A general limitation of MR is horizontal pleiotropy – where the association of a genetic variant with an outcome is independent of the exposure. “No pleiotropy” is an assumption of MR and, when violated, can make the results liable to the same errors as observational designs [30]. However, we took precautions against this by performing sensitivity analyses to detect heterogeneity, correcting for pleiotropy when outliers could not be removed, and triangulating the IVW findings. The multiple MR sensitivity estimators validated the non-null findings, and for the test of m-chronotype on MUFA, the MR-Egger estimate did not cross zero. This means that, in addition to the direction and magnitude of effect being comparable for the IVW and MR-Egger estimate, the MR-Egger estimate had enough precision to confirm the IVW finding. The different MR methods make different underlying assumptions about the nature of pleiotropy (see the “Extended Methods” section in Ref. [27]). The strongest case against pleiotropy is when none of the sensitivity estimators cross zero. It remains possible that SNPs in LD with the instruments under investigation may be associated with confounders. For instance, some of the SNPs for the MUFA instrument do affect other phenotypic traits. Thus, while we took precautions, we cannot fully rule out the possibility that causal associations we observed are attributable to other determinants.

Beneficial effects of MUFA against metabolic disorders have been documented [31,32], and the evidence from randomized-controlled trials suggests that diets rich in MUFA improve metabolic risk factors among patients with T2D [33]. Our findings validate this previous knowledge about MUFA and T2D and provide suggestive, new information about higher TOTFA and T2D, as well as m-chronotype as a factor influencing levels of Omega-3, MUFA, and TOTFA – morning chronotypes may have a predisposition towards lower levels of TOTFA and some healthy fats. Further investigation into how chronotype impacts

levels of MUFA, Omega-3, and TOTFA and teasing this out from lifestyle factors that put evening types at risk for metabolic disorders could potentially lead to population-level interventions aimed at reducing the burden of T2D.

Conflicts of interest

The authors declare no conflicts of interest.

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Data availability

All data used in this manuscript are publicly available through the MR-Base app (http://www.mrbase.org/).

Contribution statement

CDA and SLN designed the research. CDA analyzed the data and wrote the paper. SLN edited the manuscript and both authors approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.06.020.

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