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Lipidomics, atrial conduction, and body mass index: evidence from association, mediation, and Mendelian randomization models

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1 **Lipidomics, atrial conduction, and body mass index: evidence from association, mediation,**

2 **and Mendelian randomization models**

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5 Fabiola Del Greco M. PhD[1§], Luisa Foco PhD[1], Alexander Teumer PhD[2], Niek Verweij PhD[3],

6 Giuseppe Paglia PhD[1], Viviana Meraviglia PhD[4], Roberto Melotti PhD[1], Vladimir Vukovic MD,

7 PhD[1], Werner Rauhe MD[5], Peter K. Joshi MD[6], Ayse Demirkan PhD[7], Stephan B. Felix Prof,

8 MD[2], Maik Pietzner PhD[8,9], M. Abdullah Said BSc[3], Yordi J. van de Vegte BSc[3], Pim van der

9 Harst MD, PhD[3], Lifelines Cohort Study, Alan F. Wright Prof, PhD[10], Andrew A. Hicks PhD[1],

10 Harry Campbell Prof, MD[6], Marcus Dörr MD[9,11], Harold Snieder Prof, MD[12], James F. Wilson

11 Prof. PhD[6,10], Peter P. Pramstaller MD[1*§], Alessandra Rossini PhD[1*], Cristian Pattaro PhD[1*§]

12

13 *Indicates shared oversight

14 §Indicates corresponding author

15

16 **Author affiliations**

17 [1] Institute for Biomedicine, Eurac Research, affiliated to the University of Lübeck, Bolzano, Italy
18 [2] Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany
19 [3] University of Groningen, University Medical Center Groningen, Department of Cardiology, 9700 RB Groningen,
20 the Netherlands
21 [4] Department of Anatomy and Embriology, Leiden University Medical Center, Leiden, the Netherlands
22 [5] Department of Cardiology, San Maurizio Hospital, Bolzano, Italy
23 [6] Centre for Global Health Research, Usher Institute for Population Health Sciences and Informatics, University of
24 Edinburgh, Edinburgh, Scotland, United Kingdom
25 [7] Department of Human Genetics, Leiden University MC & Genetic Epidemiology Unit, Department of
26 Epidemiology, Erasmus MC, Rotterdam, the Netherlands
27 [8] Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany
28 [9] German Centre for Cardiovascular Research (DZHK), partner site Greifswald, Germany
29 [10] MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh,
30 Scotland, United Kingdom
31 [11] Department of Internal Medicine B, University Medicine Greifswald, Greifswald, Germany
32 [12] Department of Epidemiology, University of Groningen, University Medical Center Groningen, the Netherlands
33
34
35

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Authors for correspondence:

Fabiola Del Greco M, PhD

Eurac Research

Institute for Biomedicine

Via Galvani 31, 39100 Bolzano, Italy

+39 0471 055528

fabiola.delgreco@eurac.edu

Cristian Pattaro, PhD

Eurac Research

Institute for Biomedicine

Via Galvani 31, 39100 Bolzano, Italy

+39 0471 055527

cristian.pattaro@eurac.edu

Peter P. Pramstaller, MD

Eurac Research

Institute for Biomedicine

Via Galvani 31, 39100 Bolzano, Italy

+39 0471 055501

peter.pramstaller@eurac.edu

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Abstract

Background: Lipids are increasingly involved in cardiovascular risk prediction as potential pro-arrhythmic influencers. However, knowledge is limited about the specific mechanisms connecting lipid alterations with atrial conduction.

Methods: To shed light on this issue, we conducted a broad assessment of 151 sphingo- and phospholipids, measured using mass-spectrometry, for association with atrial conduction, measured by P wave duration (PWD) from standard electrocardiograms, in the Microisolates in South Tyrol study (n=839). Causal pathways involving lipidomics, BMI, and PWD were assessed using two-sample Mendelian randomization analyses based on published genome-wide association studies of lipidomics (n=4034) and BMI (n=734,481), and genetic association analysis of PWD in 5 population-based studies (n=24,236).

Results: We identified an association with relative phosphatidylcholine 38:3 (%PC 38:3) concentration, which was replicated in the Orkney Complex Disease Study (n=951), with a pooled association across studies of 2.59 (95% confidence interval: 1.3, 3.9; *P*-value=1.1×10^{-4}) *ms* PWD per *mol%* increase. While being independent of cholesterol, triglycerides, and glucose levels, the %PC 38:3 - PWD association was mediated by body mass index (BMI). Results supported a causal effect of BMI on both PWD (*P*-value=8.3×10^{-5}) and %PC 38:3 (*P*-value=0.014).

Conclusions: Increased %PC 38:3 levels are consistently associated with longer PWD, partly due to the confounding effect of BMI. The causal effect of BMI on PWD reinforces evidence of BMI's involvement into atrial electrical activity.

Introduction

P wave indices obtained from standard electrocardiograms are generally accepted as a reliable non-invasive marker of atrial conduction[1]. Among them, increased P wave duration (PWD) has been associated with cardiovascular and all-cause mortality and alterations in PWD were established as an intermediate phenotype for atrial fibrillation (AF)[1], which affects >10 million European citizens[2]. The identification of biological pathways underlying PWD regulation is important to define the physiological context of PWD within cardiovascular health.

Lipids measured using mass spectrometry are promising biomarkers for cardiovascular risk prediction[3,4]. Additionally, given the potential pro-arrhythmic effect of certain species, like in the context of ischemic heart disease, lipids are increasingly being considered as target molecules for developing new anti-arrhythmic drugs[5]. We previously measured 151 phospho- and sphingolipids in the context of the EUROSPAN consortium to conduct genome-wide association studies (GWAS) aimed at uncovering genetic loci involved in lipid regulation[6,7]. Lipids were assessed within 6 classes which included 24 sphingomyelins (SPM), 57 phosphatidylcholines (PC), 27 phosphatidylehanolamines (PE), 19 PE-based plasmalogens (PLPE), 15 lysophosphatidylcholines (LPC), and 9 ceramides (CER). The EUROSPAN GWAS identified more than thirty loci associated with individuals' lipidomic profiles and, to date, are still amongst the largest studies in the field.

To investigate whether and how lipidomics is involved in atrial conduction, we assessed whether any of such 151 phospho- and sphingolipids was associated with PWD, implementing a discovery-replication design that involved two independent population-based studies. Among the analyzed lipids, the relative concentration of PC 38:3 was strongly associated with PWD, independently of several cardio-metabolic risk factors, except for the body mass index (BMI). After investigating the mediating role of BMI, we eventually used Mendelian randomization (MR) analysis to assess causality between PC 38:3, BMI, and atrial conduction using summary statistics from the largest GWAS on lipidomics and BMI, available to date.

Methods

In accordance with Transparency and Openness Promotion Guidelines, the data that are not already included in the Data Supplement and the analytical workflow will be made available to other researchers for purposes of reproducing the results or replicating the procedure. All contributing studies received approval by their local ethics committees. All participants gave written informed consent. The full methods are available in the **Supplemental Material**.

Results

Lipidomics association analysis

By following the study workflow presented in **Figure 1**, we first carried out an observational analysis in the Microisolates in South Tyrol (MICROS) study[8,9] to assess whether any out of 151 measured lipids was associated with PWD in the general population. Results were tested for independent replication in the Orkney Complex Disease Study (ORCADES)[10]. **Table 1** outlines discovery and replication study's characteristics. MICROS participants were on average 44 years old (SD=16.1), had mean PWD=102.8 (SD=14.3) *ms*, 52% were females, 4.5% under lipid-lowering therapy (LLT), and 12.3% under anti-hypertensive treatment (AHT). ORCADES's participants were older (mean age=53, SD=15.1) and, consistently, more were under LLT (12.0%) or AHT (20.5%).

Distributions of the 151 lipids in the MICROS study are summarized in **Supplemental Table 1** and their pairwise correlations depicted in **Supplemental Figure 1**. Eighteen lipids were associated with PWD (*P*-value$\leq$0.05), including 6 PEs, 6 PCs, 3 LPCs, 2 PLPEs, and 1 SPM (**Table 2**). Replication in the ORCADES, tested at the one-sided level of 5.6×10^{-3} accounting for the 18 lipids submitted to validation, confirmed the association of PWD with %PC 38:3 (one-sided *P*-value=1.9×10^{-3}, **Table 2**). The association coefficient was very similar between MICROS (b=2.27 *ms* per *mol%* increase, SE=0.86) and ORCADES (b=3.08, SE=1.06; **Figure 2A**), with a pooled estimate β=2.59 (SE=0.67, *P*-value=1.1×10^{-4}; **Table 2**). In terms of absolute concentrations, 1 *µM* PC 38:3 increase was associated with a PWD increase of 0.06 *ms* (SE=0.02) in MICROS, and 0.07 *ms* (SE=0.04) in ORCADES, with a pooled estimate of 0.06 *ms* (SE=0.02, *P*-value=1.9×10^{-3}).

Sensitivity analysis

Results were unchanged when excluding subjects under LLT, AHT, or with a %PC 38:3 level larger than the 99th percentile from both the discovery and replication analyses (**Supplemental Table 2**). We then assessed heuristically whether the %PC 38:3 - PWD association coefficient changed when adjusting for the cardio-metabolic factors listed in **Table 3**. This was not the case for any factor, except for BMI: BMI adjustment attenuated the %PC 38:3 - PWD association to about half of the magnitude, losing significance in both MICROS (*P*-value=0.131) and ORCADES (*P*-value=0.188, **Table 3**). When fitting a multivariable model that included all quantitative cardio-metabolic risk factors together, results where similar to when adjusting for BMI only (**Table 3**). This result suggested that BMI is the only one, among the considered cardio-metabolic factors, that may have a role in the %PC 38:3 – PWD relationship, and that BMI's role is independent of that of the other factors. This motivated additional mediation and MR analyses to investigate possible causal pathways involving %PC 38:3, BMI, and PWD.

Mediation analysis

To assess whether BMI qualified as a mediator between %PC 38:3 and PWD, we conducted mediation analysis in MICROS and ORCADES separately, pooling the results via inverse-variance-weighted (IVW) meta-analysis (**Supplemental Methods**). BMI satisfied all conditions of complete mediation between %PC 38:3 and PWD in both MICROS and ORCADES (**Table 4**, upper part): %PC 38:3 was associated with BMI (*P*-value=1.0×10^{-33} and 8.5×10^{-36} in MICROS and ORCADES, respectively); PWD was associated with BMI when controlling for %PC 38:3 (*P*-value=0.016 and 2.0×10^{-4} in MICROS and ORCADES, respectively); and PWD was not associated with %PC 38:3 when controlling for BMI neither in MICROS (*P*-value=0.131) nor in ORCADES (*P*-value=0.188), except showing significance in the meta-analysis (*P*-value=0.045). Results were confirmed by the Sobel test and bootstrap analysis (**Table 4**, upper part).

When testing whether %PC 38:3 could be mediate the BMI-PWD relationship, the indirect effect of BMI on %PC 38:3 was significant in the meta-analysis (*P*-value=0.012) but not in any of the two studies (**Table 4**, lower part). BMI explained 28% of %PC 38:3 variability.

Mendelian randomization analyses

Mediation analysis results led us hypothesizing that the causal pathway could be either (1) %PC 38:3 → BMI → PWD or (2) BMI → %PC 38:3 → PWD. Therefore, we performed two-sample MR analyses to investigate the respective causal effects under the two scenarios. SNPs for use as instrumental variables (IVs) for MR analyses involving %PC 38:3 were identified from a GWAS of %PC 38:3 concentrations from the EUROSPAN consortium[6,7] (n=4034). To identify SNPs for use as IVs in MR analyses involving BMI and to obtain estimates of the association between %PC 38:3-associated SNPs and BMI, we interrogated summary data from a recent meta-analysis of GWAS of BMI from the Genetic Investigation of Anthropometric traits (GIANT) Consortium and UK Biobank (UKB) involving an average of 734,481 European-ancestry individuals[11]. SNP effect estimates on PWD were obtained by analyzing 24,236 general population individuals from the Cooperative Health Research in South Tyrol (CHRIS) study[12], Prevention of REnal and Vascular ENd-stage Disease (PREVEND)[13,14], Lifelines Cohort Study (Lifelines)[14,15], and the Study of Health in Pomerania (SHIP)[16] and SHIP-TREND[16] (**Supplemental Methods**). Characteristics of study participants (all of European ancestry) are given in **Supplemental Table 3**.

For the first hypothesized causal pathway (**Figure 3A**), we identified 3 valid IVs for %PC 38:3 from the EUROSPAN GWAS[7]: rs3198697, a missense variant of unknown clinical significance in the *PDXDC1* gene (F=100); rs968567 in *FADS2* (F=25); and rs7192552 also in *PDXDC1* (F=100) but independent of rs3198697 (LD r^2=0.008). All variants were genome-wide significant, qualified as strong instruments, and altogether explained 7% of %PC 38:3 variability (**Supplemental Table 4**). When pooling together the individual SNP MR results, we observed a non-significant positive effect of %PC 38:3 on BMI (*P*-value=0.057) with possible pleiotropy (I^2=69%, *Q* test *P*-value=0.048; **Figure 3A**). To assess the causal effect of BMI on PWD, IV selection from the GIANT+UKB meta-analysis started with all 941 genome-wide significant variants, which explained 6% of BMI variance[11]. From these, 842 SNPs qualified as strong instruments (F>10). Among them, starting from the SNP with the largest F value, we identified 187 independent (LD r^2<0.01) SNPs (**Supplemental Table 5**): these SNPs explained 2.9% of BMI variance, which is lower than the reported one, due to the more stringent selection criteria used for this MR study. Effect estimates of the 187 SNPs on PWD obtained from the meta-analysis of association results from CHRIS, PREVEND, Lifelines, SHIP, and SHIP-TREND studies (n=24,236) are shown in **Supplemental Table 5**. The combined MR estimate across all SNPs

showed a positive causal effect of BMI on PWD (P-value=1.1×10^{-3}), with no pleiotropy (I^2=0; Q test P-value=0.876; **Figure 3A**).

Next, we assessed the alternative causal pathway going from BMI to PWD through %PC 38:3 (**Figure 3B**). To assess the causal effect of BMI on %PC 38:3, we used the same 187 IVs for BMI described above and looked up the results in the EUROSPAN %PC 38:3 GWAS meta-analysis. Results supported a possible causal effect of BMI on %PC 38:3 (P-value=0.014) with limited evidence of pleiotropy (I^2=27%; Q test P-value=1×10^{-3}; **Figure 3B**; **Supplemental Table 6**). To assess the causal effect of %PC 38:3 on PWD, we tested the association of two of the SNPs identified in the EUROSPAN %PC 38:3 GWAS and PWD in the 24,236 individuals from the five studies mentioned above. SNP rs7192552 was not contained in the 1000G dataset used for genotype imputation by the five studies. There was no support for a causal effect of %PC 38:3 on PWD (P-value=0.251; **Figure 3B**; **Supplemental Table 7**).

The results of this last analysis suggest a third possible scenario where the causal effect of BMI on both %PC 38:3 and PWD, together with no causal effect of %PC 38:3 on PWD, may indicate that BMI is a confounder of the %PC 38:3-PWD association (**Figure 3C**).

Robustness of MR results was assessed in two ways. First, we performed post-hoc power calculations. Results show that our analysis would have had sufficient power to detect a causal effect of %PC 38:3 on BMI only if the causal effect had been close to the value of the association coefficient (**Supplemental Figure 2A**), that is, if the observed association was entirely due to the cause-effect relationship. Indeed, the MR effect of %PC 38:3 on BMI was ~5 times larger than the association estimate, achieving a theoretical 100% power. However, this value seems more likely to result from a small sample bias problem (small GWAS sample size; small number of instruments) and the causal effect estimate doesn't look realistic. On the other hand, our analyses were well-powered to assess a causal effect of BMI on PWD: power was already approaching 100% for causal effect estimates that were ~1/10th of the estimated association coefficient: the observed causal effect estimate fell in the 100% power range (**Supplementary Figure 2B**). Likely due to the very small association coefficient between BMI and %PC 38:3, our analysis was underpowered to assess causal effects of BMI on %PC 38:3 (**Supplementary Figure 2C**). Finally, our analysis was also underpowered to detect a causal effect of %PC 38:3 on PWD (the power for the observed MR estimate of 0.795 was 0.06; **Supplemental Figure 2D**).

Second, we re-analyzed the data using alternative two-sample MR methods such as the MR-Egger regression[17] and MR-PRESSO[18], which are more robust to pleiotropy (**Supplemental Table 8**). Being based on linear regression, they couldn't be applied to the %PC 38:3 - PWD MR analysis as only two IVs were available. For the BMI - %PC 38:3 MR analysis, MR-PRESSO confirmed the IVW result with very similar effect estimate (effect=1.2×10^{-3}, P-value=0.025). In addition, MR-Egger analysis didn't reject the hypothesis of absence of pleiotropy (intercept P-value=0.557), further supporting the validity of the IVW estimate. For the BMI-PWD MR analysis, results of the IVW were strong as they were based on several instruments and had an I^2=0 indicating no horizontal pleiotropy: in these cases, the IVW estimator is more reliable than alternative estimators. Finally, the MR-PRESSO couldn't be applied to the %PC 38:3 - BMI MR either, as the number of instruments didn't exceed the number of parameters to be estimated in the model[18]. MR-Egger results appeared unreliable, because of the low number of instruments, a 50% estimated bias, and a Q ratio of 0.1, indicating the IVW estimator as more reliable even despite large I^2.

Discussion

After screening 151 sphingo- and phospholipids for association with atrial conduction in the general population, our study identified that the relative concentration of phosphatidylcholine 38:3 was robustly associated with the duration of the electrocardiographic P wave. This association, reported here for the first time, was independent of cholesterol, triglycerides, and glycemic levels, but it was fully mediated by BMI. Additional investigations showed that BMI is causally associated with PWD in the general population, and that the %PC 38:3 - PWD association likely reflects BMI's confounding role.

Phosphatidylcholines (PC) are a class of glycerophospholipids, which are important components of biological membranes and play a fundamental role in metabolism and signaling. PC 38:3 is a combination of several isomers containing fatty acids of varying lengths and saturation attached at the C-1 and C-2 positions. Using the Lipid Maps database[19], we annotated 19 different potential PC 38:3 isomers. Based on the fatty acid composition of PC species in plasma[20], two isomers are likely to be the most abundant species in the analyzed samples, namely the PC(18:2(n-6)/20:1(n-9)) and PC(20:3(n-6)/18:0), which contain the two omega-6 fatty acids, linoleic acid

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1 (LA) (18:2(n-6)) and dihomo-γ-linolenic acid (DGLA) (20:3(n-6)). In mammals, DGLA can be

2 incorporated into cellular PCs.

3 Despite strong association, our analyses didn't show evidence of a causal link between

4 %PC 38:3 and PWD, advocating for the confounding role of BMI (**Figure 3C**). This observation

5 would be consistent with all our findings: BMI was causally associated with both %PC 38:3 and

6 PWD; the %PC 38:3 - PWD association faded away when adjusting for BMI; and no causal link

7 has been proven from %PC 38:3 to PWD. This model would suggest that increased BMI levels

8 independently cause higher circulating %PC 38:3 levels and prolonged PWD, so that the observed

9 %PC 38:3 - PWD association is just the reflection of the joint increase due to BMI. This would

10 also explain why the association of %PC 38:3 with PWD was independent of other cardio-

11 metabolic markers. The results would also be consistent with previously observed association of

12 higher PC 38:3 levels with cardiovascular disease risk[3] but not AF incidence[21].

13 The causal effect of BMI on PWD was supported by a highly powered analysis with no

14 evidence of pleiotropy. The causal effect of BMI on %PC 38:3 suffered of lack of power, which

15 suggests careful interpretation. However, while not being absent, the between-instrument

16 heterogeneity was small, giving limited room to possible pleiotropy, and the two alternative

17 estimators, IVW and MR-PRESSO, showed very similar effect estimates, which were significant

18 in both cases. On the other hand, the opposite causal effect of %PC 38:3 on BMI would receive

19 even less support from the data: the MR analysis was underpowered and based on a limited number

20 of IVs for which absence of horizontal pleiotropy could not be guaranteed.

21 Association between PC 38:3 and BMI was reported previously[22]. Our results of a causal

22 effect of BMI on %PC 38:3 might appear in contrast with a previous study that did not identify a

23 causal link between BMI and phosphatidylcholines as a whole, even though the specific

24 metabolites were not investigated[23]. For BMI to affect %PC 38:3 blood levels, one may

25 hypothesize that fat (PC 38:3) is mobilized from adipocytes. This would be inconsistent with the

26 fact that adipocytes release free fatty acids, immediately bound by serum albumin, and not PCs.

27 However, it is possible that higher BMI levels could globally and indirectly affect %PC 38:3 levels

28 through multiple signaling pathways: for instance, free fatty acids released from adipocytes might

29 be used to synthesize PCs. Because PC 38:3 can contain either DGLA or LA, the latter entering

30 human metabolism through diet only, a causal effect of PC 38:3 on BMI might look more plausible,

as such a model would be supported by established lipid metabolism physiology[24]. However, our data do not support this result.

The causal association between BMI and PWD adds to previous literature showing association between obesity and longer PWD[25] and positive association between BMI and PWD in population-based studies[26,27]. Our unconfounded MR analyses add that BMI is causally associated with PWD also in general population individuals, who were generally healthy and unselected for any disease. The causal mechanisms are probably multiple and the contrasting evidence of BMI being[28] or not[29] involved in the relationship between pericardial fat and PWD in the general population suggests that additional studies with deeply assessed clinical and molecular phenotypes are needed to disentangle the mechanisms of action of BMI on PWD. Our results recall recent evidence of a causal relationship between BMI and incident AF[30]. Together with the observation that prolonged PWD was associated with AF recurrence following cardioversion, AF following cardiothoracic surgery, and transition from paroxysmal to permanent AF[26], our additional evidence of higher BMI causing prolonged PWD calls for the need to conduct a comprehensive study of the overall causal cascade involving BMI, PWD, and (incident) AF at the general population level.

One strength of our study was the unbiased analysis of a large number of lipids and the replication of the main finding in a study population which was very different from the one used for discovery. MICROS[8,9] and ORCADES[10] were carried out in very localized contexts and different environments (the Tyrolean Alps and the Orkney Islands north of Scotland, respectively), on populations with very different cultural and life style background, leading to different health characteristics. The older age of ORCADES participants led to a longer average PWD as compared to their South Tyrolean counterparts. Despite those differences, the association coefficients between %PC 38:3 and PWD were remarkably similar. Very similar was also the attenuation of such an association due to the BMI adjustment, leading to similar results in the mediation analysis. A second strength was the standardization of the lipidomics panel across the two studies: by joining the EUROSPAN consortium[6,7], MICROS and ORCADES had lipidomics measured in the same laboratory, at the same time, and with the same method, even if on different matrixes. A third strength is the very large sample size of the GIANT+UKB meta-analysis[11] guaranteeing unbiased estimates of the causal effects of BMI on PWD and %PC 38:3. Finally, as a prerequisite of any

two-sample MR study, all studies involved were from European ancestry comparable populations with similar age distribution and from mainly population-based studies.

Some limitations could not be avoided. One aspect of concern might be the liberal significance threshold used in the discovery stage, which might have led to a high risk of type I error. We could have attained a larger sample size and set a lower significance threshold by combining MICROS and ORCADES into a more powerful single-stage design[31]. Should we have adopted such an approach, the identification of the %PC 38:3 - PWD association would have been successful as the *P*-value of the meta-analysis result was 1.1×10^{-4}: this is lower than a Bonferroni-adjusted limit of 0.05/151, that is, if we had conservatively considered all 151 lipids independent from each other. However, such a choice would have deprived us from the possibility to replicate our findings in an independent study, raising questions on the repeatability of the results. Instead, we preferred to use a discovery-replication scheme, which allowed us to verify the presence of the association in two very different studies and, at the same time, to assess the consistency of the effect estimates across studies. Such a consistency was observed at all levels: association, sensitivity, and mediation analyses. This aspect is remarkable and in our opinion is valuable at least as much, if not more, than the statistical significance in itself[32]. In any case, further confirmation of this result is warranted by subsequent studies. Another limitation is that, in MICROS, lipid-lowering and anti-hypertensive therapies could only be assessed as self-reported information during standardized interviews. This might have prevented our sensitivity analyses from observing altering effects of total-, HDL-, and LDL-cholesterol and triglycerides on the %PC 38:3 - PWD association. The absence of data on waist, hip and their ratio in the MICROS study prevented us to assess whether measures of fat distribution might also mediate the %PC 38:3 - PWD association. Further limitations concern the two-sample MR analyses. Sample overlap was between 0.5% and 2.7% of the combined exposure and outcome study sample size (**Supplemental Table 7**): as long as the IVs are strong, as in our case, such a small overlap should not bias the results[33]. A limitation of MR analyses involving %PC 38:3 as an exposure was that all three IVs might fully or in part violate the key assumption of absence of horizontal pleiotropy. The rs968567 is associated with *FADS2* and *FADS1* gene expression[34] and in strong LD with SNPs previously associated with PWD[15]. *FADS2* desaturates linoleic and alpha-linolenic essential fatty acids. This is the first step of PUFA metabolism, and also the rate-limiting, because other lipids are generated following *FADS2* activation. This is consistent with the observed associations of rs968567 with

PUFA levels[35,36], and with a vertical pleiotropy scenario, under which the instrument would still be valid. This is equally true for *FADS1* expression, acting immediately downstream *FADS2*. However, because rs968567 is also associated with inflammatory diseases[37,38], it is not possible to rule out alternative pathways from gene to atrial conduction, consistent with a possibly imbalanced horizontal pleiotropy scenario. Pleiotropy analysis is even more challenging for the *NPIPA2-MYH11* locus, containing rs3198697 and rs7192552: despite being poorly characterized, the region has been associated with lipid phenotypes by several GWAS[35,36,39,40, 41] thus making both vertical and horizontal pleiotropy two equally possible scenarios.

Conclusions

Increased levels of %PC 38:3 are consistently associated with longer PWD, reflecting the confounding role of BMI, which causally determines both. Given that both an increased BMI and a prolonged PWD are risk factors for AF, our results call further studies to assess whether a prolonged P wave duration is an intermediate causal step between increased body mass and AF.

DISCLOSURES

The authors do not have conflicts of interest.

References

1. Magnani JW, et al. P wave indices: current status and future directions in epidemiology, clinical, and research applications. *Circ Arrhythm Electrophysiol.* 2009;2:72-9.

2. Krijthe BP, et al. Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. *Eur Heart J.* 2013;34:2746-2751.

3. Stegemann C, et al. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation.* 2014;129:1821-31.

4. Razquin C, et al. Plasma lipidome patterns associated with cardiovascular risk in the PREDIMED trial: A case-cohort study 2018; *Int J Cardiol.* 2018;15;253:126-132.

5. Shao Y, et al. Lipid metabolites and their differential pro-arrhythmic profiles: of importance in the development of a new anti-arrhythmic pharmacology. *Mol Cell Biochem.* 2014;393:191-7.

6. Hicks AA, et al. Genetic determinants of circulating sphingolipid concentrations in european populations, *PLoS Genet.* 2009;5:e1000672.

7. Demirkan A, et al. Genome-wide association study identifies novel loci associated with circulating phospho- and sphingolipid concentrations. *PLoS Genet.* 2012;8:e1002490.

8. Marroni F, et al. Estimates of genetic and environmental contribution to 43 quantitative traits support sharing of a homogeneous environment in an isolated population from South Tyrol, Italy. *Hum Hered.* 2008;65:175-82.

9. Pattaro C, et al. The genetic study of three population microisolates in South Tyrol (MICROS): study design and epidemiological perspectives. *BMC Med Genet.* 2007;5:8-29.

10. McQuillan R, et al. Runs of homozygosity in European populations. *Am J Hum Genet.* 2008;83:359-372.

11. Yengo L, et al. Meta-analysis of genome-wide association studies for height and body mass index in ∼700000 individuals of European ancestry. *Hum Mol Genet.* 2018;27:3641-3649.

12. Pattaro C, et al. The Cooperative Health Research in South Tyrol (CHRIS) study: rationale, objectives, and preliminary results. *J Transl Med.* 2015;13:348.

13. Hillege HL, et al. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population, *Circulation.* 2002;106:1777-1782.

14. Verweij N, et al. Genetic determinants of P wave duration and PR segment. *Circ Cardiovasc Genet.* 2014;7:475-81.

15. Scholtens S, et al. Cohort Profile: Lifelines, a three-generation cohort study and biobank. *Int J Epidemiol.* 2015;44:1172-80.

16. Völzke H, et al. Cohort profile: the study of health in Pomerania. *Int J Epidemiol.* 2011;40:294-307.

17. Bowden J, et al. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;44:512-25.

18. Verbanck M, et al. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nature Genetics* 2018;50:693-698.

19. Sud M, et al. LMSD: LIPID MAPS structure database. *Nucleic Acids Res.* 2007;35:D527-32.

20. Žák A, et al. Fatty acid composition indicates two types of metabolic syndrome independent of clinical and laboratory parameters. *Physiol Res.* 2014;63:S375-85.

21. Ko D, et al. Metabolomic Profiling in Relation to New-Onset Atrial Fibrillation (from the Framingham Heart Study). *Am J Cardiol.* 2016;118:1493-1496.

22. Ho JE, et al. Metabolomic profiles of body mass index in the Framingham Heart Study reveal distinct cardiometabolic phenotypes. *PLoS ONE.* 2016;1:e0148361.

23. Würtz P, et al. Metabolic signatures of adiposity in young adults: Mendelian randomization analysis and effects of weight change. PLoS Med. 2014;11:e1001765.

24. Guyton AC, et al. Textbook of Medical Physiology, John E. Hall 20th edition, 2018.

25. Kosar F, et al. P-wave duration and dispersion in obese subjects. *Ann Noninvasive Electrocardiol.* 2008;13:3-7.

26. Magnani JW, et al. P wave indices, obesity, and the metabolic syndrome: the atherosclerosis risk in communities study. *Obesity (Silver Spring).* 2012;20:666-672.

27. Magnani JW, et al. Atrial electrocardiography in obesity and hypertension: clinical insights from the Polish-Norwegian Study (PONS). *Obesity (Silver Spring).* 2016;24:2608-2614.

28. Babcock MJ, et al. Pericardial fat and atrial conduction abnormalities in the Multiethnic Study of Atherosclerosis (MESA). *Obesity (Silver Spring).* 2011;19:179-184.

29. Friedman DJ, et al. Pericardial fat is associated with atrial conduction: the Framingham Heart Study. *J Am Heart Assoc.* 2014;3:e000477.

30. Chatterjee NA, et al. Genetic Obesity and the Risk of Atrial Fibrillation: Causal Estimates from Mendelian Randomization. *Circulation.* 2017;135:741-754.

31. Skol AD, et al. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet.* 2006; 38:209-13.

32. Amrhein V, et al. Scientists rise up against statistical significance. *Nature* 2019;567(7748):305-307

33. Burgess S, et al. Bias due to participant overlap in two-sample Mendelian randomization. *Genet. Epidemiol.* 2016;40:597-608.

34. Westra HJ, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet.* 2013;45:1238-1243.

35. Shin SY, et al. An atlas of genetic influences on human blood metabolites. *Nat Genet.* 2014;46:543-550.

36. Kettunen J, et al. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nat Genet.* 2012;44:269-76.

37. Okada Y, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature.* 2014;506:376-81.

38. Paternoster L, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet.* 2015;47:1449-1456.

39. Shungin D, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature.* 2015;518:187-196.

40. Randall JC, et al. Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet.* 2013;9:e1003500.

41. Willer CJ, et. al. Discovery and refinement of loci associated with lipid levels. *Nat Genet.* 2013;45:1274-1283.

1 **TABLES**

2 **Table 1.** Characteristics of the MICROS (discovery) and ORCADES (replication) study

3 samples.

		MICROS	ORCADES
Demographic characteristics	Sample size	839	951
	Age, mean(SD)	44(16.1)	53(15.1)
	Female, N(%)	435(52)	535(56)
Atrial depolarization	PWD, *ms*, mean(SD)	102.8(14.3)	110.1(18.1)
Cardio-metabolic health	BMI, kg/m^2, mean(SD)	25.4(4.6)	27.7(4.9)
	LDL, *mg/dL*, mean(SD)	135.4(42.2)	138.6(42.5)
	HDL, *mg/dL*, mean(SD)	65.1(14.5)	64.8(15.5)
	TG, *mg/dL*, mean(SD)	126.6(96.3)	120.8(79.3)
	TC, *mg/dL*, mean(SD)	226.4(46.1)	225.2(46.6)
	SBP, *mmHg*, mean(SD)	132.3(19.9)	130.3(18.8)
	DBP, *mmHg*, mean(SD)	79.5(10.8)	75.9(9.8)
	FGlu, *mmol*, mean(SD)	4.7(0.93)	5.4(1.02)
	DM, N(%)	27(3.2)	38(4.0)
Medications	LLT, N(%)	38(4.5)	113(12.0)
	AHT, N(%)	103(12.3)	195(20.5)
Absolute lipidomic concentrations	SPM	24.55(33.60)	19.79(28.49)
μM, mean(SD)	PC	44.18(104.90)	33.96(78.88)
	PE	1.41(1.94)	0.95(1.23)
	PLPE	5.83(7.11)	4.18(4.93)
	LPC	27.01(59.71)	15.47(31.87)
	CER	1.16(1.09)	0.92(0.80)
Relative lipidomic concentrations	%SPM	0.04(0.06)	0.04(0.06)
mol%, mean(SD)	%PC	0.02(0.04)	0.02(0.04)
	%PE	0.04(0.05)	0.04(0.05)
	%PLPE	0.10(0.12)	0.10(0.12)
	%LPC	0.07(0.14)	0.07(0.15)
	%CER	0.13(0.11)	0.13(0.12)

4 **Abbreviations**: SD = Standard deviation; PWD = P wave duration; AF = Atrial fibrillation; BMI = Body mass index;
5 LDL = Low-density lipoprotein cholesterol; HDL = High-density lipoprotein cholesterol; TG = Triglycerides; TC =
6 Total cholesterol; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; FGlu = Fasting glucose; DM =
7 Diabetes mellitus; LLT = lipid-lowering therapy; AHT = anti-hypertensive treatment; SPM = Sphingomyelins; PC =
8 Phosphatidylcholines; PE = Phosphatidylethanolamines; PLPE = PE-based plasmalogens; LPC =
9 Lysophosphatidylcholines; CER = Ceramides.

10

1 **Table 2.** Association analysis of PWD against relative lipidomic concentrations. The 18 lipids

2 associated with PWD with *P*-value≤0.05 in the discovery study, their replication, and discovery-

3 replication meta-analysis.

Lipid	Discovery: MICROS		Replication: ORCADES		Meta-analysis		
	b(SE)	Two-sided *P*-value	*b*(SE)	One-sided *P*-value	*b*(SE)	Two-sided *P*-value	I²%
%PE 36:3	-1.25(0.40)	1.8×10^{-3}	-0.81(0.53)	0.063	-1.09(0.32)	6.4×10^{-4}	0
%PE 34:2	-0.57(0.18)	2.1×10^{-3}	-0.23(0.22)	0.148	-0.42(0.14)	2.5×10^{-3}	30
%PE 38:4	0.55(0.19)	3.0×10^{-3}	0.07(0.20)	0.361	0.33(0.14)	0.016	67
%PE 40:6	1.05(0.37)	4.6×10^{-3}	0.35(0.28)	0.109	0.61(0.22)	6.9×10^{-3}	56
%PLPE 16:0/18:2	-1.26(0.46)	6.5×10^{-3}	-0.68(0.52)	0.097	-1.00(0.35)	3.8×10^{-3}	0
%PC 30:1	-16.32(6.01)	6.7×10^{-3}	-1.50(9.47)	0.437	-12.06(5.08)	0.017	43
%PE 40:5	3.91(1.47)	7.9×10^{-3}	0.90(1.67)	0.294	2.60(1.11)	0.019	45
%PC 38:3	**2.27(0.86)**	**8.5×10^{-3}**	**3.08(1.06)**	**1.9×10^{-3}**	**2.59(0.67)**	**1.1×10^{-4}**	**0**
%PE 38:3	3.00(1.20)	0.013	2.25(1.58)	0.078	2.73(0.96)	4.4×10^{-3}	0
%PC 38:4	0.98(0.41)	0.016	0.53(0.46)	0.125	0.80(0.30)	0.103	0
%PLPE 18:0/18:2	-0.65(0.29)	0.024	-0.51(0.31)	0.051	-0.58(0.21)	5.7×10^{-3}	0
%PC 34:2	-0.37(0.17)	0.025	-0.25(0.19)	0.093	-0.32(0.12)	0.010	0
%LPC 20:3	6.10(2.89)	0.034	0.85(2.46)	0.366	3.06(1.88)	0.102	48
%LPC 18:0	0.45(0.22)	0.042	0.59(0.26)	0.012	0.51(0.17)	2.7×10^{-3}	0
%PC 34:3	-6.84(3.41)	0.045	-1.33(3.01)	0.329	-3.75(2.25)	0.097	32
%PC 40:6	3.06(1.53)	0.045	1.53(1.11)	0.083	2.06(0.90)	0.022	0
%LPC 18:3	-16.49(8.26)	0.046	-3.87(5.91)	0.256	-8.14(4.81)	0.090	35
%SPM 18:0	1.39(0.71)	0.049	1.14(0.78)	0.072	1.28(0.53)	0.015	0

4 **Symbols/abbreviations**: *b,* estimate of the %PC 38:3-PWD association coefficient β expressing the PWD change in

5 *ms* per 1 *mol%* increase of the relative lipid concentration; SE, standard error.

6 **Boldface** highlights the results for %PC 38:3, whose association with PWD in the replication stage was robust to the

7 Bonferroni-corrected significance level of 5.6×10^{-3} (see Methods in the Supplemental Material).

8

Table 3. Analysis of potential confounders of the %PC 38:3-PWD relationship: reported are the estimated association coefficients between %PC 38:3 and PWD (b, PWD change in *ms* per 1 *mol%* PC 38:3 increase) and their standard errors (SE) when including the listed variables one at a time in the LMM.

Potential confounder	MICROS		ORCADES		Meta-analysis†	
	b(SE)	*P*-value	b(SE)	*P*-value	b(SE)	*P*-value
BMI	1.41(0.93)	0.131	1.50(1.14)	0.188	1.45(0.72)	0.045
LDL	2.13(0.93)	0.022	2.80(1.11)	0.012	2.41(0.71)	7.4×10^{-4}
HDL	2.25(0.90)	0.013	2.60(1.12)	0.020	2.39(0.70)	6.7×10^{-4}
TG	2.16(0.89)	0.015	2.92(1.11)	0.009	2.45(0.69)	4.0×10^{-4}
TC	2.11(0.88)	0.017	3.04(1.08)	5.0×10^{-3}	2.48(0.68)	2.8×10^{-4}
DBP	1.91(0.87)	0.028	2.86(1.07)	7.5×10^{-3}	2.29(0.67)	7.0×10^{-4}
SBP	1.99(0.87)	0.022	3.01(1.07)	5.0×10^{-3}	2.40(0.68)	3.9×10^{-4}
FGlu	2.11(0.87)	0.015	3.01(1.06)	5.0×10^{-3}	2.47(0.67)	2.4×10^{-4}
DM	2.32(0.86)	7.1×10^{-3}	3.11(1.07)	3.6×10^{-3}	2.63(1.32)	8.7×10^{-5}
Multivariable model*	1.45(1.04)	0.160	1.58(1.23)	0.200	1.50(0.79)	0.058

*Including all quantitative potential confounders.

†I^2 = 0% in all meta-analyses

Table 4. Mediation analysis to dissect the role of BMI in the %PC 38:3-PWD relationship.

	MICROS		ORCADES		Meta-analysis	
	b(SE)	*P*-value	b(SE)	*P*-value	b(SE)	*P*-value
Does BMI mediate the PC 38:3%-PWD relationship?						
%PC 38:3 → PWD*	2.27(0.86)	8.5×10^{-3}	3.08(1.06)	1.9×10^{-3}	2.59(0.67)	1.1×10^{-4}
%PC 38:3 → BMI (1)	3.12(0.26)	1.0×10^{-33}	3.49(0.28)	8.5×10^{-36}	3.29(0.19)	7.3×10^{-67}
(%PC 38:3 | BMI) → PWD (Direct Effect) †	1.41(0.93)	0.131	1.50(1.14)	0.188	1.45(0.72)	0.045
(BMI | %PC 38:3) → PWD (2)	0.25(0.12)	0.016	0.45(0.12)	2.0×10^{-4}	0.35(0.08)	3.7×10^{-5}
Indirect Effect = (1)×(2)	0.78(0.38)	0.040‡	1.57(0.44)	3×10^{-4}‡	1.12(0.29)	1.0×10^{-4}
Does %PC 38:3 mediate the BMI-PWD relationship?						
BMI → PWD	0.34(0.11)	0.001	0.51(0.11)	6.0×10^{-6}	0.43(0.08)	4.7×10^{-8}
BMI → %PC 38:3 (1)	$4.8 \times 10^{-4}(3.9 \times 10^{-5})$	1.2×10^{-33}	$4.0 \times 10^{-4}(3.2 \times 10^{-5})$	8.5×10^{-36}	$4.3 \times 10^{-4}(2.5 \times 10^{-5})$	2.4×10^{-68}
(BMI | %PC 38:3) → PWD (Direct Effect)	0.25(0.12)	0.016	0.45(0.12)	2.0×10^{-4}	0.35(0.08)	3.7×10^{-5}
(%PC 38:3 | BMI) → PWD (2)	1.41(0.93)	0.131	1.50(1.14)	0.188	1.45(0.72)	0.045
Indirect Effect = (1)×(2)	$1.0 \times 10^{-3}(4.5 \times 10^{-4})$	0.133§	$6.0 \times 10^{-4}(4.6 \times 10^{-4})$	0.191§	$8.0 \times 10^{-4}(4.5 \times 10^{-4})$	0.012

Abbreviations: b: estimated association coefficient; SE: standard error.
*Result reported in **Table 2**.
†Result reported in **Table 3**.
‡Sobel test *P*-values; corresponding bootstrap *P*-values: 0.04 (MICROS), 5×10^{-4} (ORCADES).
§Sobel test *P*-values; corresponding bootstrap *P*-values: 0.111 (MICROS), 0.190 (ORCADES).

FIGURE LEGENDS

Figure 1. Study flowchart.

Figure 2. Pairwise comparisons between %PC 38:3, PWD, and BMI in MICROS and ORCADES, with marginal distributions. **A**) %PC 38:3 vs PWD. **B**) %PC 38:3 vs BMI. **C**) BMI vs PWD.

Figure 3. Mendelian randomization analyses. (**A**) Pathway: %PC 38:3 → BMI → PWD. (**B**) Pathway: BMI → %PC 38:3 → PWD. (**C**) Pathways: BMI → %PC 38:3 and BMI → PWD (BMI is a confounder). **Abbreviations**: p, *P*-value; R^2_{tot}, variance explained by all IVs; F, F-statistic; *P-het*, heterogeneity Q test's *P*-value; b, effect estimate; SE, standard error. Heterogeneity summary statistics are reported for significant associations only. *5 studies: CHRIS, PREVEND, Lifelines, SHIP, and SHIP-TREND. Black and gray arrows are used to represent significant and non-significant causal associations, respectively.