Trans-fatty acid consumption and heart rate variability in two separate cohorts of older and younger adults

Luisa Soares-Miranda, BSc[1,2], **Phyllis K Stein, PhD**[3], **Fumiaki Imamura, PhD**[1], **Jacob Sattelmair, PhD**[1], **Rozenn N Lemaitre, MPH PhD**[4], **David S Siscovick, MD MPH**[4,5], **Jorge Mota, PhD**[2], and **Dariush Mozaffarian, MD DrPH**[1,6]

[1]Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

[2]Research Center in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Portugal

[3]Heart Rate Variability Laboratory, Cardiovascular Division, Washington University School of Medicine, St Louis, MO, USA

[4]Department of Medicine, Cardiovascular Health Research Unit, University of Washington, Seattle, WA, USA

[5]Department of Epidemiology, University of Washington, Seattle, WA, USA

[6]Division of Cardiovascular Medicine and Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, and Department of Nutrition, Harvard School of Public Health, Boston MA, USA

Abstract

Background—*Trans*-fatty acid (TFA) consumption is associated with risk of coronary heart disease, and *trans*-18:2, but not *trans*-18:1, in red blood cells membranes has been associated with sudden cardiac arrest. Abnormal heart rate variability (HRV) reflects autonomic dysfunction and predicts cardiac death. Relationships between TFA consumption and HRV remain under-studied. We determined whether total TFA consumption, as well as *trans*-18:1 and *trans*-18:2 TFA consumption, were independently associated with HRV in two independent cohorts in the US and Portugal.

Methods and Results—In two independent cohorts of older US adults (Cardiovascular Health Study ([CHS], age=72±5yrs, 1989/1995) and young Portuguese adults (Porto, age=19±2yrs, 2008/2010), we assessed habitual TFA intake by food frequency questionnaires in CHS (separately estimating *trans*-18:1 and *trans*-18:2) and multiple 24-hour recalls in Porto (estimating total TFA only, which in a subset correlated with circulating *trans*-18:2, but not *trans*-18:1, suggesting that we captured the former). HRV was assessed using 24-hour Holters in CHS (N=1,076) and repeated short-term (5-min) ECGs in Porto (N=160). We used multivariate-adjusted linear regression to relate TFA consumption to HRV cross-sectionally (CHS, Porto) and longitudinally (CHS). In CHS, higher *trans*-18:2 consumption was associated with lower 24-hour standard-deviation-of-all-normal-to-normal-intervals (SDNN) both cross-sectionally (−12%,

Address for Correspondence: Luisa Soares-Miranda, lsoaresm@hsph.harvard.edu. Research Centre in Physical Activity, Health and Leisure, Faculty of Sport - University of Porto, Rua Dr.Plácido Costa, 91 4200.450 Porto Portugal. Tel: +351225074785, Fax: +351225500689.

95%CI=6–19%, p=0.001) and longitudinally (−15%, 95%CI=4–25 %, p= 0.009), and lower 24-hour SDANN and SDNN-index (p<0.05 each). Higher *trans*-18:1 consumption in CHS was associated with more favorable 24-hour HRV, in particular time-domain indices (SDNN, SDANN, SDNN-index; *p*<0.05 each). In Porto, each higher SD TFA consumption was associated with 4% lower 5-min SDNN (95%CI=1–8%, p=0.04), and 7% lower 5-min rMSSD (95%CI=1–13%, p=0.04).

Conclusions—*Trans*-18:2 consumption is associated with specific, less favorable indices of HRV in both older and young adults. *Trans*-18:1 consumption is associated with more favorable HRV indices in older adults. Our results support the need to investigate potential HRV related mechanisms whereby *trans*-18:2 may increase arrhythmic risk.

Keywords

Electrophysiology; *trans*-fatty acids; heart rate variability; nutrition

INTRODUCTION

Increased dietary *trans*-fatty acids (TFA) adversely impacts cardiovascular risk factors, including markers of lipoprotein metabolism, inflammation, and endothelial function.[1–3] The magnitude of the observed associations between TFA consumption and cardiovascular disease events cannot be explained simply by changes in circulating lipids.[2, 4] Moreover, several different types of TFA exist, each with potentially different dietary sources and health effects. In particular, higher plasma phospholipid and erythrocyte membrane 18:2 TFA (*trans*-18:2), are associated with higher risks of fatal ischemic heart disease and sudden cardiac death (SCD)[5, 6] however, the latter outcome is not strongly related to blood lipid abnormalities[1]. Potential mechanisms for the relationship between TFA and SCD remain uncertain. Some have suggested that TFA may modulate cardiac membrane ion channel function[7] or have proarrhythmic properties, affecting cardiovascular electrophysiology.[8, 9] However, relationships between TFA consumption and cardiac electrophysiologic measures are not well established.

Heart rate variability (HRV) indices are established measures of cardiac electrophysiology and autonomic function. The autonomic nervous system has a central role in maintaining normal cardiac rhythm.[10] For example, lower indices of the standard-deviation-of-all-normal-to-normal-R-R-intervals (SDNN) and ultra-low-frequency power (ULF) are associated with increased risk of cardiovascular events such as myocardial infarction, cardiomyopathy, valvular heart disease, congestive heart failure and mortality.[11] Moreover, studies have suggested a relationship between lower HRV and coronary heart disease, atrial fibrillation and heart failure.[12] Furthermore, growing evidence has established heart rate (HR) as a marker of autonomic activity[13] and a higher resting HR has been associated with increased all-cause mortality, death from cardiovascular disease and SCD.[13]

Relationships between TFA consumption and HRV or HR could elucidate novel potential mechanisms whereby TFA may influence coronary heart disease and SCD risk. Relatively little is known about this topic. We tested the hypothesis that habitual TFA consumption would be associated with less favorable indices of HRV in two separate cohorts, a population-based cohort of older US adults in the Cardiovascular Health Study (CHS) and a cohort of young adults in Portugal (Porto). Given prior work that *trans*-18:2 TFA are more strongly linked to cardiac death and inflammation than *trans*-18:1 TFA[5, 6, 14–16], we hypothesized that *trans*-18:2 may be more strongly related to less favorable HRV. We therefore investigated estimated dietary consumption of *trans*-18:1 and *trans*-18:2 separately in CHS, and, in Porto in which only estimated total TFA consumption was available, we

determined whether total consumption was more closely linked to biomarkers of *trans*-18:1 or *trans*-18:2.

METHODS

Design and Samples

The study design and recruitment of CHS have been described.[17, 18] Briefly, 5,201 ambulatory, non-institutionalized men and women 65 years of age were randomly selected and enrolled from Medicare lists in 4 US communities (Forsyth County, North Carolina, Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania) in 1989–90. In 1992–93, 687 additional black participants were similarly recruited, but were not included in this analysis due to lack of baseline HRV measures. The Institutional review committee at each site approved the study, and subjects provided written informed consent. A 24-hour Holter recording was obtained at baseline (1989–90, N=1,361), and again 5 years later (1994–95) in the same subjects (N=787). We excluded participants with atrial fibrillation, flutter, or pacemakers (n=36); markedly irregular rhythms (e.g., wandering atrial pacemaker or other non-sinus rhythm) (n=48);[19] or missing data on dietary TFA consumption (N=97). Data on plasma phospholipid TFA were available in a subset of participants, measured 3 years after baseline in 1992–93. Because HRV data were not collected during that year (1992–93), prospective relationships between plasma phospholipid TFA and HRV were determined in the smaller subset of those who had valid HRV measures obtained 2 years later in 1994–95 (N=461 for time-domain and 424 for frequency-domain and non-linear measures).

The Porto cohort was established in 2008 to assess associations between lifestyle and HRV among healthy young adults.[20] We recruited university students aged 18–21 years at baseline to participate in the longitudinal study from 2008–10. After excluding 2 individuals with known cardiovascular illness or using any medication or supplements that could influence HRV or without information on TFA consumption (n=22), 160 subjects were included in the analyses. Written informed consent was obtained from participants. The study was approved by the local ethics committee and conducted in accordance to the declaration of Helsinki.

Dietary Assessment of TFA Consumption and Plasma Phospholipid

In CHS, diet was assessed at baseline (1989–90) by a validated picture-sort food frequency questionnaire (FFQ) that asked about usual dietary habits over the prior year[21], and dietary TFA consumption, including of *trans*-18:2 and *trans*-18:1 TFA separately, were estimated using the Harvard food composition database.[22] In a subset (N=146), we assessed correlations between each type of dietary TFA and plasma phospholipid levels of specific TFA measured at baseline (1989–90), as previously described.[22]

In Porto, we assessed total TFA consumption using an annual 24-hour dietary recall performed each year over 3 years. Portion sizes of food and drink consumed were estimated using food models and photos. Food consumption was converted to nutrient values, including total TFA consumption and energy intake, by *Food Processor Plus*®, (ESHA Research, Oregon, USA) which uses the United States Department of Agriculture database.[23] Traditional Portuguese dishes were added using Portuguese food composition databases.[24] Due to limited food composition data, estimates of TFA consumption in Porto were only available for total TFA and not for different types of TFA. We therefore evaluated correlations between total TFA consumption and specific plasma phospholipid TFA in a subset (N=40) to identify which type(s) of TFA was being captured by the Porto dietary estimates.

Assessment of HRV

HRV can be evaluated by time-domain, frequency-domain and non-linear methods[25] and based on either short-term (e.g., 5 to 20-minutes) or long-term (e.g., 24-hour) recordings (Table 1). Short-term measures obtained at rest do not capture circadian or sleep-related changes and reflect mainly resting parasympathetic (respiratory) variation in HR. Long-term measures reflect a complex interaction of autonomic inputs, and can also capture longer-term circadian differences in HRV as well as daytime and night-time baroreceptor and respiratory autonomic variation.

HRV assessment in CHS[28, 29] and Porto[20] have been described previously. In CHS, a 2-channel 24-hour Holter recording was obtained (Del-Mar-Medical Systems, Irvine, California), and 24-hr HR and HRV were determined at the Washington University School of Medicine HRV laboratory. Recordings were acceptable for analysis with 18 hours of usable data, requiring for time-domain analyses 50% of each segment to be N-N (normal-to-normal) interbeat intervals (n=1076 in 1989–90; n=578 in 1994–95) and for frequency-domain and nonlinear analyses, which are more sensitive to missing data, 80% of each segment to consist of N-Ns interbeat intervals (n=1034 in 1989–90; n= 544 in 1994–95). Beat onset detection and classification were reviewed and edited by trained technicians and overread in detail by Dr. Stein (GE Marquette-Mars 800-Holter analyzer, Milwaukee, Wisconsin). In Porto, R-R intervals were recorded during 20-minutes in a quiet room using the Polar-Advantage NV Heart Monitor (Polar-Electro-OY, Finland), which in healthy subjects provides R-R interval measurements comparable to more conventional ECG devices.[30, 31] During the last 5-minutes of recording, used to analyze HRV, participants were in the supine position and matched their breathing to a metronome-paced frequency of 12 breaths/min. R-R intervals were analyzed using Kubios-HRV Software-1.1.[32]

Covariates

In CHS, participants completed a standardized questionnaire on medical history, health status, and lifestyle habits and underwent a clinic examination including: blood pressure, ECG and anthropometric measures.[17] Possible dietary confounders were estimated from responses to the FFQ. Usual leisure-time physical activity was assessed at baseline (1989–90) and at the third (1992–93) annual visit using a modified Minnesota Leisure-Time Activities questionnaire that evaluated frequency and duration of fifteen different activities during the prior two weeks.[33]

In Porto, anthropometric measures were obtained by standardized methods at each of three visits. Habitual alcohol intake (yes/no) and smoking habits (yes/no) were assessed annually by questionnaire. Potential dietary confounders, such as omega-3 polyunsaturated fatty acids, fiber, and total energy intake, were estimated from the multiple 24-hour recalls. Free-living physical activity was objectively monitored for seven days by uniaxial accelerometers (model-GT1M, Fort Walton Beach, Florida). Freedson's cutoffs were used to analyze accelerometer data via a Mahuffe-activity analyzer[34], and daily-time spent in moderate-to-vigorous physical activity was calculated.

Statistical Analysis

HRV measures were tested for normality through numeric and graphical methods (swilk and qnorm commands in STATA), and natural log transformed as needed to facilitate parametric comparisons. In CHS, cross-sectional associations at baseline between estimated *trans*-18:1 and *trans*-18:2 consumption and HRV were evaluated by multivariate-adjusted linear regression. We also evaluated prospective associations between TFA consumption at baseline and HRV at the fifth visit. Additionally, we analyzed relationships between plasma phospholipid TFA measured at the third annual visit and HRV measured at the fifth annual

visit by multivariate-adjusted linear regression. Dietary and plasma phospholipid TFA were normalized to one standard deviation (SD) differences for mutual comparison. Multivariate models were adjusted for: age, gender, race, education, income, clinical sites, smoking, body mass index (BMI), prevalent diabetes mellitus, coronary heart disease, hypertension, β-blocker use or anti-hypertensive medication, leisure-time physical activity, alcohol, total energy intake, energy adjusted eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), 16:1 TFA (*trans*-16:1), energy adjusted quintiles of fruit and energy adjusted quintiles of vegetable consumption. We additionally assessed fiber consumption; levels of fibrinogen, C-reactive protein, triglycerides, LDL cholesterol, and HDL cholesterol; and use of digitalis, anti-arrhythmic medication, and anti-depressants as potential confounders or mediators. Inclusion of these variables did not appreciably alter results, and thus these were not included in the final analyses. Missing covariate values (all<8%) were imputed by linear regression using age, race, gender, income, and prevalent cardiovascular disease.

In Porto, we took advantage of repeated measures of diet and HRV (2008-09-10) by evaluating random-effects linear regression models, simultaneously assessing the multiple measures and taking into account within-individual variability, and also by evaluating simple linear regression using the averages of the three measures of TFA consumption (g/day) and HRV to minimize within-person measurement error. Both methods provided similar results, and we present the results based on their averages. We used multivariate-adjusted linear regression to examine cross-sectional associations over three years between total TFA consumption and HRV as continuous variables, normalizing the TFA measure to one SD for comparability. Multivariate models were adjusted for: age, total energy intake, gender, smoking, physical activity, alcohol intake, and BMI. We examined other potential confounders or mediators, including fiber intake, blood glucose and triglycerides, but these were not included in the final model, as they did not alter results. Missing accelerometer values for physical activity (8%) were imputed with a linear regression using nonmissing data from the other visits, as well as age and gender. TFA consumption in each cohort was adjusted for total energy intake using the residual method.[35] Potential nonlinear associations between TFA consumption and SDNN were assessed using restricted cubic splines.[36] All *p* values were two-tailed (α=0.05). Analyses were performed using Stata-10.1 (College-Station, Texas).

RESULTS

Table 2 shows the descriptive characteristics of both cohorts. At baseline, CHS participants were 72±5 years old, with average total TFA consumption of 3.7±1.2 grams/day. In Porto, at baseline participants were 19±2 years old, with average total TFA consumption of 1.6±1.5 grams/day.

Cross-sectional Associations between TFA consumption and HRV at baseline in CHS

In CHS, after multivariate adjustment, increased *trans*-18:2 consumption was cross-sectionally associated with decreases in several indices of 24-hour HRV (Table 3). Among time-domain measures, each one SD (0.06 g/day) of higher *trans*-18:2 consumption was associated with 12% lower SDNN and lower standard-deviation-of-5-minute-average-N-N-intervals (SDANN) (both reflecting long-term circadian HRV), and 11% lower SDNN-index (average-of-the-5-min-standard-deviations-of-N-N-intervals, reflecting combined sympathetic and parasympathetic modulation of HR) (p<0.01 each). Consistent with this, among frequency-domain measures, each one SD higher *trans*-18:2 consumption was associated with lower circadian HRV and vagal modulation as reflected by 24% lower total power (TP) and ultra-low-frequency power (ULF), and 24% lower very-low ultra frequency power (VLF) (p<0.01 each). *Trans*-18:2 consumption was also associated with higher HR,

including both 24-hour average HR (+3.20 bpm/SD of consumption, p=0.006) and resting HR (+3.26 bpm/SD of consumption, p=0.01).

In contrast to findings for *trans*-18:2, *trans*-18:1consumption at baseline was cross-sectionally associated with higher HRV (Table 3). One SD (0.68 g/day) of higher *trans*-18:1 consumption was associated with 16% higher SDNN and SDANN and 14% higher SDNN-index (p<0.01 each) and 37% higher TP and ULF, 33% higher VLF (p<0.01 each). *trans*-18:1 consumption was also associated with lower HR (24-hour HR: −2.85 bpm/SD, p=0.02; resting HR: −3.09 bpm/SD, p=0.02).

Neither *trans*-18:2 nor *trans*-18:1 consumption were associated with other HRV indices, including rMSSD (square-root-of-the-mean-of-the-squares-of-successive-N-N differences; reflecting mainly vagal modulation of HR), low-frequency high-frequency power ratio (LF/HF), normalized low-frequency power (LFnu), normalized high-frequency power (HFnu), Poincaré plot ratio (SD12), nor the short-term fractal scaling exponent (DFA1).

Longitudinal Associations between TFA consumption at baseline and HRV 5 years later in CHS

Longitudinal analyses in CHS (Table 4) were generally consistent with the cross-sectional analyses. Greater *trans*-18:2 consumption was associated with lower time-and frequency-domain circadian and vagal indices of HRV measured 5 years later, whereas higher *trans*-18:1 consumption was associated with several more favorable indices of HRV. Each one SD higher consumption of *trans*-18:2 at baseline was associated with lower time-domain measures 5 years later, including 15% lower SDNN and SDANN and 14% lower SDNN-index (p<0.05 each); and a trend toward higher resting HR (+3.88 bpm, p=0.06). Conversely, each one SD higher *trans*-18:1 consumption at baseline was associated with higher time-domain measures 5 years later, including: 19% higher SDNN and SDANN and 15% higher SDNN-index, (p<0.05 each); and a trend toward lower resting HR (−4.11 bpm, p=0.05). One SD higher *trans*-18:2 consumption was associated with 30 % lower TP and ULF (p<0.05 each). In contrast, one SD higher *trans*-18:1 consumption was associated with 44% higher TP and 45% higher ULF (p<0.05 each). Neither *trans*-18:2 nor *trans*-18:1 were prospectively associated with rMSSD, LF/HF, HFnu, LFnu, VLF, nor non-linear indices of HRV.

In a subset of 146 CHS participants with phospholipid fatty acid measures at the same time as the dietary assessments, estimated dietary consumption of each TFA was moderately correlated with the respective plasma phospholipid levels (*r*=0.21 for *trans*-18:1; *r*=0.30 for *trans*-18:2). Based on linear regression between different reported foods and dietary *trans*-18:2 consumption in CHS, the unit of *trans*-18:2 consumption evaluated in the present analysis (one SD, 0.06 mg/d) corresponded to about 1 serving/day of baked foods (doughnuts, cookies, cakes, pastry) or 1 serving/day of commercial fried foods.

Longitudinal Associations between plasma phospholipid TFA levels in year 3 and HRV 2 years later in CHS

We investigated relationships between plasma phospholipid TFA levels at year three and HRV two years later in CHS (N=461) (Table 5). Higher levels of plasma phospholipid *trans*-18:1 were associated with higher time-domain HRV measures including 5% higher SDNN (p=0.006) and 6% higher SDANN (p=0.006); higher frequency-domain measures including 10% higher TP and 11% higher ULF (p=0.01 each); and lower resting HR (−1.49, p=0.02). Additionally plasma phospholipid *trans*-18:1 levels were associated with more favorable non-linear indices including higher DFA1 (+0.03 higher, p=0.01) and lower SD12

(4% lower, p=0.03). Levels of plasma phospholipid *trans*-18:2 were not significantly associated with HRV indices in the longitudinal analysis.

Sensitivity Analyses in CHS

Age-adjusted analyses were generally similar to the multivariate analyses (supplementary material). In sensitivity analysis, when excluding subjects with prevalent CHD at baseline in CHS or adjusting for waist circumference in place of BMI, results were also generally similar (data not shown). In post-hoc analyses, we investigated potential interaction between the TFA subtypes by evaluating the trans-18:2/trans-18:1 ratio. No significant independent association of this ratio with any HRV index was observed (data not shown).

Cross-sectional Associations between TFA consumption and HRV in Porto

Only total TFA consumption was measured in Porto, estimated from multiple 24-hour recalls. In a subset (N=40), total TFA consumption correlated with plasma phospholipid *trans*-18:2 (r=0.32, p=0.04), but not with *trans*-18:1 (r=0.02, p=0.80). This suggested that total TFA consumption in Porto, as estimated by the dietary recall and the food composition database in this cohort, largely reflected consumption of *trans*-18:2, rather than consumption of *trans*-18:1. After multivariate adjustment, total TFA consumption was cross-sectionally associated with lower 5-min HRV in Porto (Table 6). Each 1 SD (1.5 g/d) of higher TFA consumption was related to lower values of time-domain indices, including 4% lower SDNN and 7% decreased rMSSD (p=0.04 each). Consistent with these results, TFA consumption was also associated with a trend toward higher HR (+1.10 bpm, p=0.07), and lower HF (−11%, p=0.08) (also reflecting vagally-mediated HRV).

Linearity vs. non-linearity of relationships between TFA consumption and HRV

We found little evidence for nonlinearity of observed relationships. For example, in Porto higher total TFA consumption was monotonically associated with lower SDNN, and in CHS, higher *trans*-18:2 consumption was monotonically associated with lower SDNN (Figure 1).

DISCUSSION

Among older adults in CHS, higher *trans*-18:2 consumption was both cross-sectionally and prospectively associated with specific, less favorable indices of HRV. Consistent with these findings, in Porto, we observed cross-sectional associations of total TFA consumption, which correlated most strongly with circulating phospholipid *trans*-18:2, with short-term HRV indices assessed annually over 3 years. To our knowledge, this study is the first to identify, in two independent cohorts, a relationship between habitual dietary consumption of TFA, particularly *trans*-18:2, and unfavorable HRV measures. These results support emerging evidence that *trans*-18:2, in particular, may increase cardiac risk.[1–3, 5, 6]

TFA consumption was only associated with certain HRV indices. In CHS, *trans*-18:2 consumption was associated with less favorable indices that reflect 24-hour circadian activity as SDNN, SDANN, TP, and ULF, as well as with less favorable indices that mainly reflect vagal modulation, such as HR. Additionally, *trans*-18:2 consumption was associated with lower values of VLF power. Although the exact physiologic mechanisms responsible for VLF power are still a matter of discussion, evidence suggests that VLF may reflect both vagal control of heart rate; the activity of the renin-angiotensin system[11]; and it is also related with HR and coefficient of variation, which may reflect functional capacity.[26, 27]*Trans*-18:2 consumption was also associated with higher 24-hour and resting HR. In contrast, *trans*-18:2 consumption was not significantly associated with other HRV indices, including measures of erratic vs. more organized HRV patterns (DFA1, Poincaré

ratio)[19] or indices such as LFnu, HFnu, and LF/HF that can be interpreted as reflecting relative sympathetic modulation, relative parasympathetic modulation of HR or sympathovagal balance respectively.[11] Similarly, in Porto, TFA consumption was inversely associated with specific supine short-term measures of HRV, including SDNN, rMSSD, and a trend toward higher HR, but not with other measures.

Lower SDNN and ULF are significant predictors of clinical events including: myocardial infarction, cardiomyopathy, mortality, and arrhythmic mortality.[11] Moreover, lower rMSSD, VLF and higher HR may suggest a relative reduction in parasympathetic activity.[11] Loss of protective vagal reflexes appears related to ventricular tachyarrhythmias.[37, 38] Furthermore, some evidence suggests that vagal activity may contribute to immune responses and lowering of inflammation, e.g., the "nicotinic anti-inflammatory pathway".[39, 40] Higher resting HR is also an independent risk factor for SCD, fatal cardiovascular disease, and all-cause mortality.[13] We recognize that while HRV indices are associated with risk of clinical events and specifically with SCD, their sensitivity and specificity for risk of malignant arrhythmias are not high. Relatively few clinical characteristics or diagnostic tests have high sensitivity or specificity for risk of significant arrhythmias, including most traditional cardiac risk factors for many of which associations with life-threatening arrhythmias are weaker than for HR and HRV. For example, among 8,917 middle aged (35 to 69 years) Japanese adults, lower HRV was more strongly associated with risk of SCD (RR=2.01, 95% CI=1.17, 3.44, for higher vs. lower HRV evaluated as a binary variable) than several traditional cardiovascular risk factors including: total cholesterol (RR=0.85, 95% CI= 0.50, 1.44, per 10 mg/dl), triglycerides (RR=0.99, 95%CI= 0.94, 1.05, per 10 mg/dl), and BMI (RR=1.01, 95%CI= 0.93, 1.09, per 1kg/m^2)[41]. In the present analysis, the associations of dietary TFA with HRV may help elucidate potential pathways of effects of TFA consumption on the heart.

Our findings support further experimental investigation of how fatty acids in general, and TFA in particular, might affect cell membrane and ion channel functions. In vitro and animal studies suggest that dietary fatty acids can alter the function of *trans*-membrane cell proteins, including cardiac ion channels.[7] Specific individual fatty acids appear to be preferentially incorporated into lipid rafts or caveolae that modulate membrane receptor function.[7] In a small (N=79) 8-week intervention study, where a diet rich in TFA was given to one group of men[42], a *post hoc* analysis suggested that daily 20g TFA dietary supplementation tended to reduce rMSSD and increase HR, but conclusions were limited by the small sample size, the exclusion of several subjects from the final analysis, and the relatively high HRV in these healthy men. Also, TFA consumption in this study (6.8% of total energy) was from bakery products and comprised both *trans*-18:1 and *trans*-18:2 (55% and 5% of total fatty acids, respectively), limiting conclusions for specific effects of different TFAs. Our findings support the possibility of adverse HRV effects of *trans*-18:2 consumption at usual dietary levels of intake in free-living populations.

The associations of dietary *trans*-18:2 with HRV in CHS could not be confirmed using plasma phospholipid TFA. Reasons for this inconsistency are unclear. Biomarker levels were available in fewer subjects, which could have limited statistical power to detect associations. Dietary questionnaire and circulating biomarker values of TFA are also each imperfect estimates of true habitual TFA consumption. Circulating levels reflect the *in vivo* balance of both diet and metabolism, rather than dietary consumption alone; and also reflect relatively short-term (several weeks) exposure. Conversely, dietary questionnaires estimate TFA consumption with errors due to variability in TFA content of otherwise very similar appearing foods, product formulations over time, and relative lack of comprehensive nutrient databases on TFA levels, especially *trans*-18:2 levels, in multiple categories of foods. Thus, given their different sources of errors, the observed correlations between the

TFA dietary estimates and biomarker levels in our cohorts represent underestimates of their association with true long-term TFA consumption. Notably, because each of these sources of error are unlikely to be related to HRV, these errors limit the ability to detect associations with HRV. Consequently, the actual associations of TFA consumption with specific HRV indices may be stronger than we observed, and the other null findings we observed, both for dietary TFA and TFA biomarkers, should be interpreted with caution.

We estimated that one SD of *trans*-18:2 consumption in CHS corresponded to about 1 servings/day of bakery foods or 1 serving/day of commercial fried foods. Trans-18:1 is the most abundant type of TFA in foods made with partially hydrogenated vegetable oils.[22] In contrast, many known food sources of partially hydrogenated vegetable oils do not correlate with *trans*-18:2 blood levels, suggesting that blood levels may also be determined by exposure from other potential dietary sources, e.g. possibly related to oil deodorization or high temperature cooking processes.[22] Our findings support the need for further investigation of potential electrophysiologic effects of dietary *trans*-18:2 and determinants of both their dietary exposure and circulating blood levels.

We found dietary and plasma phospholipid *trans*-18:1 to be associated with more favorable HRV measures in CHS, including indices of abnormal HR patterns (DFA1 and SD12), circadian modulation (SDNN, SDANN, ULF), vagal activity (VLF, HR). The divergent associations with HRV of *trans*-18:2 versus *trans*-18:1 are consistent with emerging evidence that these fatty acids may have different effects on some health outcomes. In our own and others' previous work, *trans*-18:2 has had generally positive associations, while *trans*-18:1 has had generally null or inverse associations, with risk of coronary heart disease and sudden cardiac arrest.[5, 6, 8, 16, 43] A recent animal study, focusing on atherosclerosis, found that *trans*-18:2 consumption increased biomarkers of endothelial dysfunction (ICAM-1) and oxidative stress.[9] However, the potentially greater harm of *trans*-18:2 versus *trans*-18:1 cannot be stated with certainty. Even though some studies support this concept[5, 6, 8, 16, 43], others have not found consistent differences in associations with cardiac risk of *trans*-18:1 versus *trans*-18:2 fatty acids.[8, 14, 15] Differences in health effects of *trans*-18:1 and *trans*-18:2 deserve further attention, especially as most policy focus to-date has been on partially hydrogenated vegetable oils that contain mostly *trans*-18:1.

Our analysis had several strengths. We evaluated the relationships between TFA consumption and HRV, including both short-term and 24-hour indices, in two separate cohorts. Information on dietary habits, HRV measures, and others risks were collected prospectively by standardized methods. We evaluated several HRV using several classes of measures including time-domain, frequency-domain and non-linear. We adjusted for several relevant confounders characterized prospectively using standardized methods, minimizing residual confounding. We found generally similar findings for *trans*-18:2 consumption in older and younger adults in two distinct cohorts employing different methods for dietary and HRV assessments, which increases confidence in the validity and generalizability of our findings.

Potential limitations are acknowledged. In both cohorts, residual confounding due to unknown or incompletely measured factors cannot be excluded, even though a range of covariates were available and evaluated as potential confounders. Inconsistency between CHS results for dietary and plasma phospholipid *trans*-18:2 was present. The 2 cohorts were different in many ways, including country, subject age, time period of evaluation, and dietary and HRV assessment methods. For example, given the age differences in these cohorts, the participants in CHS may have been exposed to TFA for a longer period of time than those in Porto. Similarly, types and sources of TFA exposure may not have been the same in these two cohorts. Nevertheless, in light of these many differences, we found

generally concordant results in these two independent cohorts. Our findings cannot distinguish potential acute vs. chronic relationships between TFA and HRV, and further investigation of time courses of potential effects is needed. We evaluated multiple HRV indices, increasing the possibility of a chance finding. However, the outcomes of the analyses were generally consistent in the two cohorts and with our prespecified hypotheses.[44] The Porto cohort had a smaller sample size and potential for measurement error from using 24-hour dietary recalls to assess TFA consumption, which could have attenuated findings toward the null. Conversely, the serial assessments over three years increased statistical power and would reduce error inherent in 24-hour recalls. Most participants were Caucasian, potentially limiting generalizability if effects of TFA consumption on HRV vary by race.

In conclusion, estimated *trans*-18:2 consumption was associated with specific and less favorable indices of HRV in both young and older adults, whereas *trans*-18:1 consumption and blood levels were positively associated with some HRV indices in older adults. Even with broad differences in age ranges, other cohort characteristics, and diet and HRV assessment methods, results were generally consistent in both studies. Because HRV is a measure of autonomic function, which is a potential mediator for heart disease and especially SCD, our results indicate the need for further studies to characterize the dietary sources and potential electrophysiological roles of different TFA subtypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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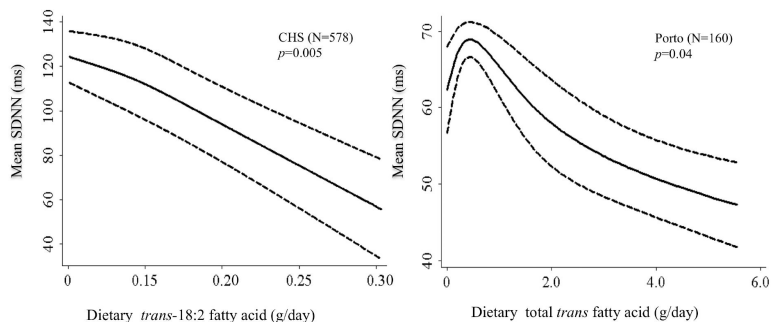
Figure 1.

Multivariable-adjusted associations of *trans*-fatty acid intake and mean standard deviation of the N-N intervals (SDNN), as assessed nonparametically by means of restricted cubic splines. As expected due to differences between 24-hour vs. short-term (5-min) HRV, SDNN mean values were higher in CHS than in Porto.

Values in CHS are adjusted for age (years), gender (male/female), race (white/nonwhite), education (<high school, high school, >high school), income (/>$25,000), clinical sites (four categories), smoking (never/former/current), BMI (kg/m^2), diabetes mellitus (yes/no), coronary heart disease (yes/no), hypertension (three categories), β-blocker use (yes/no), other anti-hypertensive medication (yes/no), leisure-time physical activity (kcal/week), alcohol use (drinks/week), and consumption of total energy (kcal/d), *trans*-16:1 fatty acids (mg/day), EPA and DHA (quintiles), fruits (quintiles), and vegetables (quintiles). Values in Porto are adjusted for age (years), gender, current smoking (yes/no) moderate to vigorous physical activity (min/day), alcohol use (yes/no), BMI (kg/m^2), and consumption of total n-3 PUFA (mg/day), dietary fiber (g/day) and total energy (kcal/d).

Table 1

Measures of heart rate variability (HRV) in the Porto and Cardiovascular Health Study cohorts.[*]

Variable	Brief Physiologic Correlation
Analysis of Short-term recordings (5-min)	
Time-domain	
SDNN, ms	Combined sympathetic and parasympathetic modulation of the heart rate due to respiratory sinus arrhythmia, representing cardiac vagal control. When measured in a supine position with paced breathing, 5-min SDNN mostly reflects parasympathetic activity. Higher values reflect higher parasympathetic (vagal) influence.
rMSSD, ms	Mainly parasympathetic modulation of the heart rate due to respiratory sinus arrhythmia, representing cardiac vagal control. Higher values reflect higher parasympathetic (vagal) influence.
Frequency-domain	
HF, ms^2	Vagal modulation of heart rate in response to respiration. Higher values reflect higher parasympathetic (vagal) influence or greater beat-to-beat variability due to erratic rhythm.
Analysis of long-term recordings (24-hour)	
Time-domain	
SDNN, ms	Standard deviation of all N-N intervals (from the entire recording). Reflects longer-term circadian differences and total HRV.
SDANN, ms	Standard deviation of 5-min average of N-N intervals. Total circadian activity. Additionally, it is related with HR and coefficient of variation, which may reflect functional capacity[26,27].
SDNN-index, ms	Averaged 5-min SDNN. Reflects combined sympathetic and vagal activity but independent of circadian rhythm.
rMSSD, ms^2[†]	Root mean square of successive differences between N-N intervals. Reflects the average of daytime and night-time parasympathetically-mediate respiratory variation. Higher values reflect higher parasympathetic (vagal) influence or greater degree of erratic rhythm[19]
Frequency-domain	
LF/HF	Has been proposed has an index of sympathovagal balance. However interpretation of this index is controversial.
LFnu, %[†]	Precise interpretation of this index is controversial. However there is evidence that normalized LF can be a measure of sympathetic modulation of heart rate. LF band is between 0.04 and 0.15 Hz.
HFnu, %[†]	Relative vagal modulation of heart rate in response to respiration. Higher values reflect higher parasympathetic (vagal) influence or greater degree of erratic rhythm. HF band is between 0.15 and 0.4 Hz,
ULF, ms^2	Fluctuations in R-R intervals with underlying cycle length of >5-min and 24-hour. Predominantly circadian rhythm but other influences including activity and neuroendocrine rhythms. ULF band is below 0.003Hz. Additionally, it is related with HR and coefficient of variation, which may reflect functional capacity[26,27].
TP, ms^2	Variance of all N-N intervals.
VLF, ms^2	VLF may reflect both vagal control of heart rate and also the effect of the renin-angiotensin system.
Non-linear	
DFA1	Short-term fractal scaling exponent. Reflects randomness or correlatedness of the N-N intervals pattern. Totally random R-R intervals pattern has a value of 0.5, whereas a totally correlated pattern has a value of 1.5.
SD12, Poincare Ratio	Organization of heart rate patterns based on the ratio of the axes of an ellipse fitted to the scatter plot of N-N vs. N-N+1 intervals. Higher values can reflect a greater degree of erratic rhythm.

DFA1= short-term fractal scaling exponent, HF= high-frequency power, HFnu =normalized high-frequency power; SD12= Poincaré plot ratio, rMSSD=square-root-of –the-mean-of-the-squares-of-successive-R-R-intervals differences, SDNN=standard-deviation-of-the-R-R-intervals, SDANN= standard-deviation-of-5-minutes-average-of-the-R-R-intervals, SDNN-index, TP= total power, ULF= ultra-low-frequency power, VLF= very low frequency power.

*
In Porto, frequency domain HRV was assessed using a traditional fast Fourier transform. Repeated HRV measures in Porto had high intracorrelation (Cronbach's Alpha: SDNN 0.83; rMSSD 0.84, HF 0.74; HF 0.82), suggesting good internal consistency. In CHS, the sampling frequency was 128 Hz, and ectopic beats were handled by linear spline, although ectopy was not highly prevalent in this population-based rather than clinical cohort.

†
Specific HRV indices including rMSSD, LFnu, and HFnu can be exaggerated by erratic HR patterns, i.e., sinus arrhythmia of non-respiratory origin, which is especially common in older adults. Therefore, to minimize confounding, by erratic rhythm in CHS, we assessed rMSSD, LFnu, and HFnu among individuals with lower erratic HRV (DFA1>1.04 for baseline analyses and DFA1>1.14 for longitudinal analyses), consistent with our prior methods for HRV analyses in this cohort.[28] Sensitivity analyses evaluating rMSSD, LFnu, and HFnu among all participants were not significantly different.

Table 2

Baseline characteristics of younger adults in the Porto and older adults in the Cardiovascular Health Study.

Characteristic	Porto	Cardiovascular Health Study
N	160	1,076
Age, years	19±2	72±5
Gender, % male	48	46
Race, % white	100	95
Education, % greater or equal to high school	100	75
Body mass index, kg/m^2	23±3	27±4
Prevalent diabetes mellitus, %	0	15[*]
Prevalent coronary heart disease, %	0	24[*]
Prevalence of hypertension, %	0	40[*]
Current smoking, %	6	10
Leisure-time activity, kcal/wk	[†]	1392±1747[†]
Physical activity, min/day	61±29[†]	[†]
β blocker use, %	0	14
Anti-hypertensive medication use, %[*]	0	43[*]
Estimated dietary intake		
Total energy intake, g/d	2169±622[‡]	2029±684[§]
Total *trans*-fatty acids intake, g/d	1.6±1.5[‡]	3.7±1.2[§]
Trans-18:1, g/d	[‡]	2.0±0.7[§]
Trans -18:2, g/d	[‡]	0.16±0.06[§]
Trans -16:1, g/d	[‡]	0.08±0.03[§]
Total n-3 PUFAs, g/d	0.66±0.4[‡]	0.28±0.2[§]
HRV indices		
SDNN, ms	67.1±29.9[//]	121.6 ±34.8[¶]
SDANN, ms	[//]	110.8±33.4[¶]
SDNN-índex, ms	[//]	44.1 ±15.2[¶]
rMSSD, ms	72.1±40.7[//]	26.5±16.9[¶]
TP, ln ms^2	[//]	9.50±0.59[¶]
ULF, ln ms^2	[//]	9.37±0.61[¶]
HF, ln ms^2	7.84±0.85[//]	4.44±0.73[¶]
DFA1	[//]	1.04±0.18[¶]
SD12	[//]	0.27±0.1[¶]
HR, bpm	69.0±9.1[//]	73.3 ±9.0[¶]

Values are mean±standard deviation (continuous variables) or percentage (categorical variables). For more details on these HRV indices, see Table 1.

[*]Diabetes =fasting glucose >140 mg/dl, two hour post-oral challenge glucose >200 mg/dl, or use of insulin or oral hypoglycemic medications. Coronary heart disease=history of myocardial infarction, angina, or coronary revascularization. Hypertension = three categories: (1) systolic <140 and diastolic <90 mm Hg; (2) systolic 140–159 or diastolic 90–94 mmHg; and (3) systolic 160+ or diastolic 95+ mm Hg or if the participant report physician-diagnosed hypertension and also taking antihypertensive medication. The prevalence of the third category is reported here. Anti-hypertensive medication use included calcium-channel blockers, diuretics, vasodilators, or angiotensin converting enzyme inhibitors.

[†]Estimated in Porto by accelerometer and in CHS by the Minnesota Leisure-Time Activities questionnaire.

[‡]Estimated using the average of three 24-hour recalls. Individual *trans* fatty acids not separately estimated in Porto.

[§]Estimated using a semi-quantitative food frequency questionnaire.

[//]Estimated using short-term (5-min) measures; longer-term HRV indices not available in Porto

[¶]Estimated using long-term (24-hour) measures.

Table 3

Multivariate-adjusted cross-sectional differences in heart rate and heart rate variability (HRV) per each one standard deviation higher intake of *trans*-18:2 and *trans*-18:1 among older adults in the Cardiovascular Health Study.

	Trans-18:2 intake		*Trans*-18:1 intake	
	Multivariate-adjusted difference (95%CI) in each HRV index for each 1 SD (0.06 g/d) higher consumption of *trans*-18:2 fatty acids[*]	p	Multivariate-adjusted difference (95%CI) in each HRV index for each 1 SD (0.68 g/d) higher consumption of *trans*18:1 fatty acids [*]	p
24-hour Heart Rate, bpm[†] (N=1,076)	+3.20 (0.92, 5.48)	0.006	−2.85 (−5.17, −0.52)	0.02
Resting Heart Rate, bpm[†] (N=1,076)	+3.26 (0.68, 5.83)	0.01	−3.09 (−5.71, −0.46)	0.02
Time-domain HRV (N=1,076)				
SDNN, ms	−12% (−19, −6)	0.001	+16% (7, 25)	<0.001
SDANN, ms	−12% (−19, −5)	0.001	+16% (7, 26)	<0.001
SDNNindex, ms	−11% (−19, −3)	0.007	+14% (4, 24)	0.004
rMSSD, ms[‡]	−9% (−19, 4)	0.17	+7% (−4, 21)	0.27
Frequency-domain HRV (N=1034)				
TP, ms^2	−24% (−36, −11)	0.001	+37% (16, 60)	<0.001
ULF, ms^2	−24% (−36, −11)	0.001	+37% (16, 61)	<0.001
VLF, ms^2	−24% (−36, −9)	0.003	+33% (11, 60)	0.002
LF/HF, ratio	−1% (−14, 15)	0.96	+1% (−13, 17)	0.90
LFnu, % [†‡]	+1.09 (−1.04, 3.24)	0.32	−0.4 (−2.56, 1.79)	0.73
HFnu, % [†‡]	−1.16 (−2.81, 0.5)	0.17	+0.65 (−1.03, 2.33)	0.45
Non-linear HRV (N=1034)				
DFA1[†]	−0.01 (−0.05, 0.04)	0.77	+0.01 (−0.04, 0.06)	0.73
SD12	0% (−8, 8)	0.91	+1% (−8, 8)	0.95

Analyses adjusted for: age (years), gender (male/female), race (white/nonwhite), education (< high school, high school, > high school), income (/ > $ 25,000/yr), clinical site (four categories), smoking (never/former/current), BMI (kg/m^2), diabetes mellitus (yes/no), coronary heart disease (yes/no), hypertension (three categories), β-blocker use (yes/no), other anti-hypertensive medication use (yes/no), leisure-time physical activity (kcal/week), alcohol (drinks/week), and consumption of total energy (kcal/d), *trans*-16:1 fatty acids (mg/day), EPA and DHA (quintiles), fruits (quintiles), and vegetables (quintiles). All models also mutually adjusted for consumption of *trans*-18:2 and *trans*-18:1 to investigate their independent effects.

In sensitivity analyses, results for pNN50 were generally similar to those for rMSSD; and we found no significant associations of TFA with heart rate turbulence (HRT).

R-squared values are the following: 0.21 (24-hour HR); 0.19 (resting HR); 0.15 (SDNN); 0.15 (SDANN); 0.14 (SDNNindex); 0.10(rMSSD); 0.14 (TP); 0.15 (ULF); 0.17 (VLF); 0.17 (LF/HF ratio); 0.13 (LFnu); 0.16 (HFnu); 0.17 (DFA1); 0.14 (SD12). nu = normalized units

[*] Because most HRV indices were log-transformed prior to analysis, values represent the percent difference in each HRV index according to each one unit (one SD) of higher TFA consumption.

[†] Normally distributed and were not log-transformed prior to analysis. Values represent absolute difference in each HRV index according to each one unit (one SD) of higher plasma phospholipid TFA.

[‡] Because erratic (abnormal sinus) HRV is common in older adults and can bias certain HRV indices, rMSSD, LFnu, and HFnu were evaluated among individuals with lower erratic HRV (DFA>median; n =625), consistent with our prior methods for HRV analyses in this cohort.[28]

Table 4

Multivariate-adjusted longitudinal differences in heart rate and heart rate variability (HRV), 5 years after dietary assessment, per each one standard deviation higher intake of *trans*-18:2 and *trans*-18:1 fatty acids among older adults in the Cardiovascular Health Study.

	Trans-18:2 intake		*Trans*-18:1 intake	
	Multivariate-adjusted difference (95%CI) in each HRV index for each 1 SD (0.06 g/d) higher consumption of *trans*-18:2 fatty acids*	*p*	Multivariate-adjusted difference (95%CI) in each HRV index for each 1 SD (0.68 g/d) higher consumption of *trans*-18:1 fatty acids*	*p*
Resting Heart Rate, bpm (N=529) †	+3.88 (−0.10, 7.86)	0.06	−4.11 (−8.18, −0.44)	0.05
Time-domain HRV (N=578)				
SDNN, ms	−15% (−25, −4)	0.009	+19% (5, 34)	0.007
SDANN, ms	−15% (−25, −3)	0.02	+19% (5, 36)	0.009
SDNNindex, ms	−14% (−25, −1)	0.03	+15% (1, 32)	0.04
rMSSD, ms‡	−10% (−36, 11)	0.34	+12% (−9, 38)	0.26
Frequency-domain HRV (544)				
TP, ms²	−30% (−46, −10)	0.005	+44% (11, 86)	0.006
ULF, ms²	−30% (−47, −9)	0.008	+45% (11, 90)	0.007
VLF, ms²	−23 (−41, 2)	0.07	+24% (−7, 64)	0.14
LF/HF, ratio	10% (−12, 38)	0.38	−7% (−26, 17)	0.56
LFnu, %†‡	0.69 (−2.66, 4.04)	0.68	−0.04 (−3.46, 3.38)	0.98
HFnu, %†‡	−1.37 (−4.22, 1.48)	0.34	+1.14 (−1.77, 4.04)	0.77
Non-linear HRV (N=544)				
DFA1†	+0.01 (−0.07, 0.09)	0.79	−0.005 (−0.09, 0.08)	0.90
SD12	−3% (−17, 7)	0.57	+3% (−8, 18)	0.60

See Legend Table 3.

‡ rMSSD, nuLF, and nuHF were evaluated among individuals with lower erratic HRV (DFA> median; n=345). R-squared values are the following: 0.15 (resting HR); 0.12 (SDNN); 0.11 (SDANN); 0.12 (SDNNindex); 0.16(rMSSD); 0.13 (TP); 0.12 (ULF); 0.17 (VLF); 0.16 (LF/HF ratio); 0.15(LFnu); 0.18 (HFnu); 0.17 (DFA1); 0.13 (SD12).

Table 5

Multivariate-adjusted longitudinal differences in hear rate and heart rate variability (HRV) per each one standard deviation higher levels of plasma phospholipid *trans*-18:2 and *trans*-18:1 fatty acids assessed two years earlier in the Cardiovascular Health Study.

| | Plasma phospholipid *trans*-18:2 | | Plasma phospholipid *trans*-18:1 | |
	Multivariate-adjusted difference (95%CI) in each HRV index for each 1 SD (0.08 g/d) higher of plasma phospholipid *trans*-18:2 fatty acids[*]	*p*	Multivariate-adjusted difference (95%CI) in each HRV index for each 1 SD (0.71 g/d) higher of plasma phospholipid *trans*-18:1 fatty acids[*]	*p*
Resting Heart Rate, bpm (N=454)[†]	+0.87 (−0.26, 2.00)	0.13	−1.49 (−2.70, −0.28)	0.02
Time-domain HRV (N=461)				
SDNN, ms	+1% (−3, 4)	0.73	+5% (2, 9)	0.006
SDANN, ms	+1% (−3, 5)	0.62	+6% (2, 10)	0.006
SDNNindex,ms	−1% (−5, 3)	0.69	+4% (−1, 8)	0.10
rMSSD, ms[†]	−1% (−7, 7)	0.98	−3% (−10, 4)	0.44
Frequency-domain HRV (N=424)				
TP, ms^2	+1% (−8, 8)	0.99	+10% (2, 19)	0.01
ULF, ms^2	+1% (−7, 8)	0.98	+11% (2, 21)	0.01
VLF, ms^2	−3% (−11, 5)	0.48	+9% (0, 19)	0.05
LF/HF, ratio	+1% (−5, 8)	0.72	+7% (−1, 15)	0.06
LFnu, %[†‡]	−0.12 (−1.15, 0.93)	0.83	+0.60 (−0.44, 1.63)	0.26
HFnu, %[†‡]	−0.39 (−1.26, 0.47)	0.37	−0.48 (−1.34, 0.39)	0.28
Non-linear HRV (N=424)				
DFA1[†]	−0.008 (−0.033, 0.017)	0.51	+0.03 (0.008, 0.06)	0.01
SD12	0% (−4, 4)	0.87	−4% (−8, 0)	0.03

Analyses adjusted for: age (years), gender (male/female), race (white/nonwhite), education (< high school, high school, > high school), income (/ > $ 25,000), smoking (never, former, current), BMI (kg/m^2), diabetes mellitus (yes/no), coronary heart disease (yes/no), hypertension (three categories), β-blocker use (yes/no), anti-hypertensive medication use (yes/no), leisure-time physical activity (kcal/d), alcohol use (drinks/week), and plasma phospholipid levels of *trans*-16:1 fatty acids, *trans*-18:1, *trans*-18:2, EPA and DHA. *Trans*-18:1 and *trans*-18:2 fatty acids were mutually adjusted.

In sensitivity analyses, results for pNN50 were generally similar to those for rMSSD; and we found no significant associations of TFA with heart rate turbulence (HRT).

R-squared values are the following: 0.15 (resting HR); 0.09 (SDNN); 0.09 (SDANN); 0.09 (SDNNindex); 0.12(rMSSD); 0.10 (TP); 0.10 (ULF); 0.13 (VLF); 0.15 (LF/HF ratio); 0.18(LFnu); 0.25 (HFnu); 0.15 (DFA1); 0.09 (SD12). nu = normalized units

[*] Because most HRV indices were log-transformed prior to analysis, values represent the percent difference in each HRV index according to each one unit (one SD) of higher plasma-phospholipid TFA.

[†]Normally distributed and were not log-transformed prior to analysis. Values represent absolute difference in each HRV index according to each one unit (one SD) of higher plasma phospholipid TFA.

[‡]Because erratic (abnormal sinus) HRV is common in older adults and can bias certain HRV indices, rMSSD, nuLF, and nuHF were evaluated among individuals with lower erratic HRV (DFA> median; n =265), consistent with our prior methods for HRV analyses in this cohort.[28]

Table 6

Multivariate-adjusted cross-sectional differences in heart rate and heart rate variability per each one standard deviation higher intake of total *trans*-fatty acids among younger adults in Porto.

	Total *trans*-fatty acids intake Multivariate-adjusted difference (95%CI) in each HRV index for each 1 SD (1.5 g/d) higher consumption of total *trans*-fatty acids[*]	*p*
Resting Heart rate, bpm (N=160)[†]	+1.1 (−0.7, 2.3)	0.07
Time-domain HRV (N=160)		
SDNN, ms	− 4% (−8, −1)	0.04
rMSSD, ms	−7%(−13, −1)	0.04
Frequency-domain HRV (N=160)		
HF, ms^2	−11% (−22, 2)	0.08

R-squared values are the following: 0.36 (resting HR); 0.14 (SDNN); 0.15 (rMSSD); 0.13 (HF)

[*] Because most HRV indices were log-transformed prior to analysis, values represent the percent difference in each HRV index according to each one unit (one SD) of higher total TFA consumption.

[†] Resting HR was normally distributed and not log-transformed prior to analysis. Thus, these values represent the absolute difference in resting HR according to each one unit (one SD) of higher total TFA consumption. Analysis adjusted for age: (years), gender, current smoking (yes/no) moderate to vigorous physical activity (min/day), alcohol use (yes/no), BMI (kg/m^2), and consumption of total n-3 PUFA (mg/day), dietary fiber (g/day) and total energy (kcal/d).