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1 **Fatty acid desaturase genetic variations and dietary omega-3 fatty acid intake associate with**
 2 **arterial stiffness**

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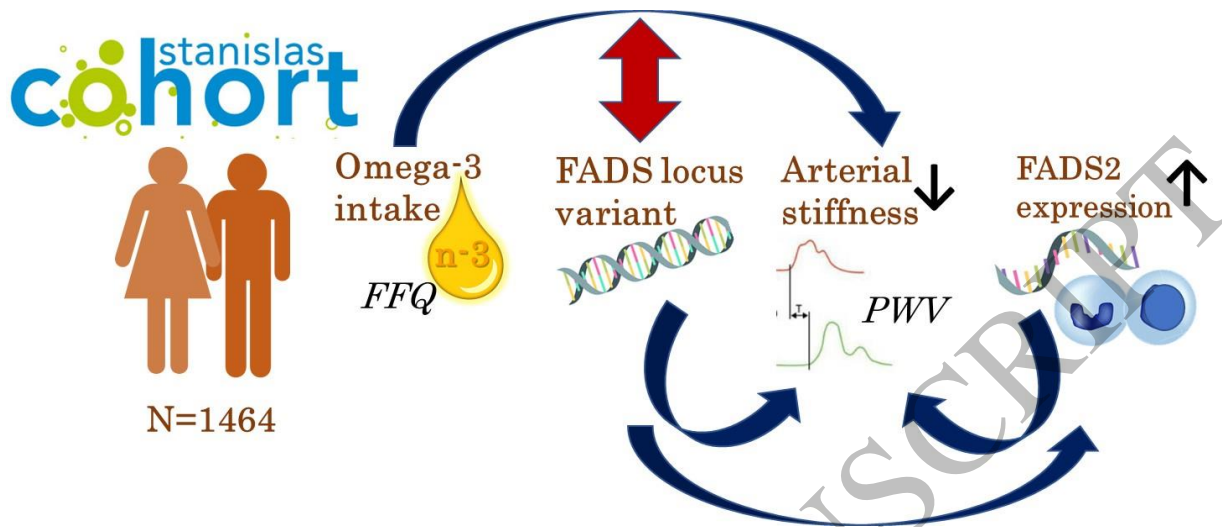
1 **Abstract**

2 **Background** Long-chain polyunsaturated fatty acids (PUFAs) generate diverse bioactive lipid
3 mediators, which tightly regulate vascular inflammation. The effects of omega-3 PUFA
4 supplementation in cardiovascular prevention however remains controversial. In addition to
5 direct dietary intake, fatty acid desaturases (FADS) determines PUFA levels. Increased arterial
6 stiffness represents an independent predictor of mortality and cardiovascular events. The aim
7 of the present study was to determine the association of PUFA intake, FADS1 genotype, and
8 FADS expression with arterial stiffness. **Methods** Cross-sectional population-based cohort study
9 of 1464 participants without overt cardiovascular disease. Dietary intake was assessed using a
10 food frequency questionnaire. Arterial stiffness was assessed by carotid femoral pulse wave
11 velocity (PWV) and FADS1 locus variant was determined. Blood cell transcriptomics was
12 performed in a subset of 410 individuals. **Results** PWV was significantly associated with the
13 FADS1 locus variant. Differential associations between PWV and omega-3 PUFA intake were
14 observed depending on the FADS1 genotype. High omega-3 PUFA intake attenuated the FADS1
15 genotype-dependent associations. Carriers of the minor FADS1 locus variant exhibited
16 increased expression of FADS2, which associated with PWV. **Conclusions** Taken together, these
17 findings point to FADS1 genotype-dependent associations of omega-3 PUFA intake on
18 subclinical cardiovascular disease. These findings may have implications for identifying
19 responders and non-responders to omega-3 PUFA supplementation and open up for
20 personalized dietary counselling in cardiovascular prevention.

21 **Keywords:** Arterial stiffness, Dietary, Inflammation, Omega-3 fatty acids, Prevention, STANISLAS
22 cohort

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4

Graphical Abstract
165x68 mm (1.8 x DPI)

5 Introduction

6 Long-chain polyunsaturated fatty acids (PUFAs) generate diverse bioactive lipid mediators,
 7 which tightly regulate inflammatory processes. The omega-6 PUFA arachidonic acid (AA) is the
 8 precursor for proinflammatory lipid mediators, *e.g.* 4-series leukotrienes and 2-series
 9 prostaglandins, formed through lipoxygenase and cyclooxygenase activities, respectively. When
 10 using the omega-3 PUFA eicosapentaenoic acid (EPA) as substrate, the latter enzymatic
 11 pathways generate 5-series leukotrienes and 3-series prostaglandins, which are less biologically
 12 active and hence act as anti-inflammatory mediators by competition with the AA pathway.
 13 Furthermore, EPA and docosahexaenoic acid (DHA) are metabolized into specific lipid
 14 mediators involved in the resolution of inflammation¹. These include resolvins, maresins and
 15 protectins, collectively referred to Specialized Proresolving Mediators (SPMs)² which act as
 16 “stop-signals” in vascular inflammation³ with beneficial effects in models of *e.g.* atherosclerosis,
 17 intimal hyperplasia and vascular calcification⁴.

18 In the randomized controlled trial (RCT) REDUCE-IT, a high dose of EPA (4 g)
 19 provided a 25% risk reduction for cardiovascular events⁵. Other recent RCTs of omega-3 PUFA
 20 supplementation have however been neutral for the primary cardiovascular outcomes^{6, 7},
 21 raising the notion that omega-3 PUFA formulations, doses and the relative EPA and DHA
 22 content may be decisive cardiovascular prevention effects of omega-3 PUFAs⁸, which is

1 supported by a recent meta-analysis⁹. In addition, cardiovascular risk reduction by omega-3
2 PUFA supplementation is more pronounced in subjects with low basal dietary omega-3 PUFA
3 intake⁶, supporting that the overall omega-3 PUFA exposure is of importance for cardiovascular
4 health.

5 Increased arterial stiffness, measured as carotid-femoral pulse wave velocity
6 (PWV), represents an independent predictor of mortality and cardiovascular events¹⁰. An
7 increased PWV occurs as a result of ageing and/or subclinical pathophysiological process in the
8 vascular wall, in which chronic non-resolving inflammation may play a key role^{3, 10}. Whereas
9 higher circulating omega-3 PUFAs are associated with lower PWV^{11, 12}, results from studies
10 examining omega-3 PUFA intake and arterial stiffness are conflicting^{13, 14}. Data from large
11 population-based cohorts are hence needed for determining if dietary contribution of omega-3
12 PUFAs is sufficient to induce beneficial effects on arterial stiffness.

13 In addition to direct dietary intake, fatty acid desaturases (FADS) 1 and 2 catalyzes
14 an endogenous synthesis of AA and EPA from dihomo- γ -linolenic acid and eicosatetraenoic acid,
15 respectively. Genome-wide association studies have identified single-nucleotide polymorphisms
16 (SNPs) in the FADS1 gene that are associated with FADS expression and that determines fatty
17 acid composition of phospholipids¹⁵. A Mendelian randomization study recently showed that
18 the associations of fatty acids with risk of 15 cardiovascular diseases were driven by the intronic
19 SNP rs174547 in FADS1, with the minor allele predicting a lower cardiovascular risk¹⁶. The
20 association between FADS1 locus variant and PWV has been reported in a limited cohort of
21 obese individuals¹⁴, but no previous study has evaluated the association between FADS1
22 genotype, PUFA intake and PWV in a large population, nor as a measure of subclinical vascular
23 alterations in the absence of overt cardiovascular disease. Whereas several studies have shown
24 that variations within the FADS1 gene influence PUFA levels following fish oil supplementation
25 or dietary interventions¹⁷⁻¹⁹, less is known about a possible gene-diet interaction for FADS1 with
26 habitual dietary omega-3 PUFA intake.

27 Based on the above, the aim of the present study was to determine the association
28 of PUFA intake, FADS1 rs174547 genotype, and FADS gene expression with PWV.

29

1 **Methods**

2 **Study Population**

3 Participants attending the fourth visit of the longitudinal familial cohort STANISLAS (Suivi
4 Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux)²⁰ were included in this
5 study. The STANISLAS cohort is a population-based study of 1006 families that each comprise 2
6 parents and at least 2 children (4295 participants) from the Lorraine region (eastern France)
7 recruited during 1993–1995 at the Center for Preventive Medicine. The participants were of
8 French origin and free of acute or chronic disease. 1705 participants underwent the fourth
9 examination from 2011 to 2016. After exclusion of participants with missing information on
10 food intake (n=10), without subclinical organ damage assessment data (n=56), cardiovascular
11 disease history (n=26), and genetic data (n=86) as well as those with a daily energy intake either
12 below 1000 or above 5000 kcal (n=63), the cross-sectional study population who underwent at
13 the fourth examination consisted of 1464 participants. 410 individuals had available
14 transcriptomic data and were included in the gene expression analysis (Supplementary Figure
15 1). The research protocol was conducted according to the Declaration of Helsinki and was
16 approved by the local ethics committee (Comité de Protection des Personnes Est III, Nancy,
17 France). All study participants gave written informed consent to participate.

18 **PUFA and dietary intake assessment**

19 Dietary intake was assessed using a validated food frequency questionnaire (FFQ) as previously
20 described^{21, 22}. In brief, the participants reported their consumption frequency and portion size
21 of 133 food items over the previous 3 months. The consumption frequency was reported using
22 6 levels in the questionnaire, ranging from “never or rarely” to “twice or more a day.” The
23 portion size of each food item was estimated using standard serving sizes and food models.
24 PUFAs intake, i.e. DHA, EPA, docosapentaenoic acid (DPA), linoleic acid (LA), alpha linolenic acid
25 (ALA) and AA were estimated from all the items in a scale of grams per day by multiplying the
26 consumption frequency of each item by the nutrient content of selected portions. Nutritional
27 data were extracted from the French food composition database established by the French
28 Data Centre on Food Quality (Ciqual, last updated in 2013). The ratio omega 6/omega 3 was
29 calculated as follow: $(AA+LA)/(EPA+DHA+DPA+ALA)$.

1 **Carotid-femoral pulse-wave velocity**

2 The carotid femoral PWV was measured using the Complior device (Alam Medical, France) in a
3 quiet room after at least 10 minutes of rest in supine position according to the
4 recommendations of the European Network for the Noninvasive Investigation of Large
5 Arteries^{20, 23}. Two sensors were placed simultaneously on the carotid artery and on the femoral
6 artery. Two measurements were made, with cfPWV calculated as their mean. If the 2
7 measurements differed by >0.5 m/s, a third measurement was made, and the cfPWV was then
8 calculated as the median of the 3 measurements. The onboard foot-to-foot algorithm based on
9 the second-derivative waveforms was used to determine the transit time. The carotid-to-
10 femoral, carotid-to-sternal-notch, and sternal-notch-to-carotid distances were measured with a
11 measuring tape. The distance used for cfPWV calculation was 0.8 times the direct carotid-
12 femoral distance. cfPWV was calculated as distance divided by transit time.

13 **Genotyping**

14 Genotyping was performed as previously described²⁴. In brief, whole blood DNA was extracted
15 using Genra Puregene Blood Kit (Qiagen, Hilden, Germany) and stored at -20°C. Genotyping
16 was conducted at the Centre National de Recherche en Génomique Humaine (Evry, France)
17 using two DNA chips from Illumina (Exome Array and Global Screening Array) and data for the
18 *FADS1* SNP rs174547 was extracted for 1611 individuals after quality control steps (callrate
19 100% and HWeq=0.06).

20 **FADS gene expression**

21 Whole blood RNA was extracted from PAXgene Blood RNA Tubes (Qiagen, Hilden, Germany)
22 using MagMAX for Stabilized Blood Tubes RNA Isolation Kit (Life Technologies, Villebon sur
23 Yvette, France) on a King Fisher Duo Prime automate (ThermoFisher Scientific, Dardilly, France).
24 Extracted RNAs were quantified using a Nanodrop spectrophotometer and the quality was
25 assessed using the RNA ScreenTape system (Agilent, les Ulis, France). Transcriptome analysis
26 was conducted at IMoPA (Vandoeuvre-les-Nancy, France) using Clariom D[®] assays (Affymetrix,
27 ThermoFisher Scientific, Dardilly, France). The differential expression of the *FADS1* and *FADS2*
28 genes was assessed in a subgroup of 410 individuals. Briefly, the analysis of differential gene
29 expression according to rs174547 genotype, was carried out with the Transcription Analysis

1 Console Software 4.0.2, including SST-RMA intensity normalization followed by statistical gene
2 expression analysis with the Limma R package (e-Bayes method), with a statistical threshold
3 (FDR < 0.05). Gene expression values are referring to normalized intensities values (in log₂)
4 obtained from Clariom D[®] microarrays for each individual. Quantitative gene expression value
5 from normalized intensities is expressed and plotted as Normalized Intensity Units (N.I.U.).
6 Mann-Whitney-Wilcoxon test was applied for comparison of means of normalized intensities,
7 between the appropriated rs174547-genotype groups.

8 **Covariates**

9 A self-reported questionnaire was used to collect demographic and socioeconomic information,
10 such as age, sex, education level, smoking status, and physical activity as well as information
11 about disease history and treatment²⁵. Anthropometric measurements such as weight, height,
12 and waist circumference (WC) were recorded at clinical examination. Blood samples were
13 collected, and the serum concentrations of the following biomarkers were measured: fasting
14 glucose, high-density lipoprotein cholesterol (HDL-C), and triglycerides. Office blood pressure²⁰
15 and 24-hour ambulatory blood pressure^{26, 27} were measured. In brief, participants underwent
16 24-hour recording of the ambulatory blood pressure using the Spacelabs 90207 ambulatory
17 monitor (Spacelabs Medical), with the monitoring cuff placed around the non-dominant arm.
18 The blood pressure system was programmed to make measurements every 15 minutes from 6
19 AM to 10 PM and every 30 minutes from 10 PM to 6 AM. Metabolic Syndrome was defined
20 according to National Cholesterol Education Program ATP3²⁸ as the presence of ≥3 of the
21 following components: elevated WC (>102 cm for men, >88 cm for women); elevated
22 triglycerides (≥1.5 g/L) or treated by lipid-lowering drugs; reduced HDL-C (<0.4 g/L for men, <0.5
23 g/L for women); elevated office systolic blood pressure (SBP, ≥130 mm Hg) or elevated diastolic
24 blood pressure (DBP, ≥85 mm Hg), or treated by antihypertensive drugs; or elevated glucose
25 (≥1.10 g/L) or treated by antidiabetic drugs. Elevated triglycerides was defined as ≥1.5 g/L or
26 treated by lipid-lowering drugs.

27 **Statistics**

28 Participant characteristics were stratified by sex and compared using a chi-square test for
29 categorical variables and either a Student's t test or a Wilcoxon nonparametric tests for

1 continuous variables. PUFA and energy intake by FADS1 rs174547 genotype were compared
2 using ANOVA. Mixed models were performed to assess the associations between EPA or DHA
3 intake and PWV. A random effect on family was added in the models to take into account the
4 familial link between some individuals. Adjustment variables were selected after testing the
5 associations between the covariates and PWV (Supplementary Table 1). Only variables with
6 $p < 0.05$ were retained: age, sex, BMI, systolic blood pressure (SBP). As the PUFA were highly
7 correlated ($r = 0.99$, $p < 0.0001$), adjustment for other PUFA than the one tested in the analyses
8 was not performed. Third, mixed models were used as described above to assess the
9 associations between rs174547 FADS1 locus variant and PWV. Additive and recessive models
10 were performed as follows, for the additive model, homozygotes for minor allele (CC) were
11 noted 0, heterozygotes (CT) were noted 1 and homozygotes for the major allele (TT) were
12 noted 2. For the recessive model, CT and TT genotypes were grouped together and noted 1 as
13 previously described²⁹. Genotypes were also used for stratification, according to additive and
14 recessive models. Sensitivity analyses were performed after using stratification by sex and PUFA
15 intake. The associations between FADS1 and FADS2 expression with PUFA intake and/or PWV
16 were assessed using a mixed model, as described above. Statistical analyses were performed
17 with SAS version 9.4 (SAS Institute, Cary, NC).

18 **Results**

19 **Fatty acid intake**

20 The characteristics of the study population according to sex are shown in Table 1. The overall
21 PUFA intake was higher in men compared with women and represented a higher proportion of
22 total energy intake in women compared with men (Table 1). The median overall omega-6 to
23 omega-3 intake ratio was almost 2-fold above the recommended 4:1, with higher ratio in men
24 compared with women. The FADS1 locus variant had the expected distribution¹⁶ and was not
25 significantly different across sexes (Table 1). There were no significant differences in PUFA
26 intake by FADS1 locus variant (Supplementary Table 2).

27 **Association of EPA and DHA intake with pulse wave velocity**

28 In age- and sex-adjusted analyses, DHA and EPA were negatively associated with PWV at the
29 limit of significance (Supplementary Table 3, β (95%IC): -0.39(-0.78;-0.007); -0.56(-1.13;0.01),

1 respectively; $P=0.05$ for both). Further adjustment for SBP, BMI, and energy intake attenuated
2 the observed associations to not reaching statistical significance (β (95%CI): -0.46 (-1.07 ; 0.15)
3 , $p=0.14$ for EPA, and -0.32 (-0.73 ; 0.09) for DHA, $p=0.13$). Given the differences in PUFA intake
4 and PWV between men and women in the present cohort (Table 1), further analyses were
5 performed by sex. The inverse association of PWV with DHA and EPA were significant in males
6 (β : -0.70 (-1.36 ; -0.04); $P=0.04$ for DHA, and -1.01 (-1.99 ; -0.03); $P=0.04$ for EPA) but not in
7 females (β : -0.16 (-0.60 ; 0.29); $P=0.49$ for DHA, and -0.23 (-0.89 ; 0.43); $P=0.49$ for EPA) in the
8 age- adjusted analysis but these associations were non-significant in both sexes after further
9 adjustment for BMI, SBP, and energy intake (males: -0.64 (-1.34 ; 0.06), $p=0.07$; females:
10 -0.09 (-0.56 ; 0.37), $p=0.69$ for DHA; males: -0.92 (-1.97 ; 0.13), $p=0.08$, females: -0.15 (-0.84 ;
11 0.54), $p=0.67$ for EPA).

12

13 **FADS1 locus variant confers lower pulse wave velocity**

14 PWV was significantly associated with the FADS1 locus variant (β (95% CI): 0.15 (0.04 ; 0.27);
15 $P=0.007$), with lowest PWV in carriers of the minor C-allele. The association remained
16 significant after adjustments for BMI and SBP (Table 2). Adjusting on energy intake or each
17 PUFA did not change the association. The significant association between the FADS1 locus
18 variant and PWV observed in the age and sex adjusted analysis (Table 2) was reproduced only in
19 men in a sex-stratified analysis (β (95% CI): 0.20 (0.01 ; 0.38); $P=0.03$). In contrast, no significant
20 association was observed in females (β (95% CI): 0.10 (-0.03 ; 0.23); $P=0.13$). These sex-
21 dependent differences were retained in models adjusting for age, BMI, SBP and PUFA intake
22 (Supplementary Table 4).

23 **Association of fatty acid intake with pulse wave velocity according to FADS1 locus variant**

24 The association of fatty acid intake with PWV after stratification according to genotype are
25 shown in Figure 1 (EPA Fig 1A and 1B and DHA Fig 1C and 1D). The projection of the estimation
26 after linear regression analysis was adjusted on either sex and age (Model 0; Figure 1A and 1C)
27 or on sex, age, BMI, and SBP (Model 1; Figure 1B and 1D). The results indicated a recessive
28 pattern for the minor allele with similar associations in the CT and TT genotypes, whereas the
29 associations between intake of either EPA or DHA with PWV were largely attenuated in

1 homozygous C allele carriers (Fig 1). Grouping the CT and TT genotypes revealed a significant
2 negative association for DHA (β -0.42 (-0.83; -0.01); $P=0.04$) and a borderline negative
3 association for EPA (β -0.61 (-1.22 ; 0); $P=0.05$) with PWV in the age- and sex-adjusted analysis.
4 These associations were somewhat attenuated after further adjustments for BMI and SBP (β -
5 0.41 (-0.82; 0); $P=0.05$ for DHA, and β -0.60(-1.23 ; -0.01); $P=0.06$ for EPA). In contrast, no
6 significant associations for either DHA or EPA intake were observed in the CC genotype in crude
7 (β 0.02 (-1.23; 1.27), $p=0.98$ for DHA; β 0.04(-1.81;1.89), $p=0.96$ for EPA) or adjusted models (β
8 0.30(-1.01 ; 1.61), $p=0.66$ for DHA; β 0.44 (-0.87; 1.75), $p=0.66$ for EPA). There were however no
9 significant interactions between genotype and fatty acid intake in any of the models.

10 **Association of FADS1 locus variant with pulse wave velocity according to fatty acid intake**

11 Stratifying the population based on the median into high and low dietary PUFA intake revealed
12 a significant association between FADS1-rs174547 genotypes and PWV in individuals with low
13 intake of EPA, and DHA and a low w6/w3 ratio (Table 3). These associations remained
14 significant after further adjustment for BMI and SBP. In contrast, for subjects with a dietary
15 intake of EPA, DHA and ratio w6/w3 above median, no significant associations for the genotype
16 were observed.

17 **FADS2 gene expression according to FADS1 locus variant and pulse wave velocity**

18 Since PWV significantly associated with the FADS1 rs174547 genotype (β 0.15 (0.04 ; 0.27);
19 $P=0.007$), and FADS1 locus variant only associated with FADS2 expression, associations of
20 FADS1 and FADS2 expression levels with PVW were subsequently assessed.

21 The age, sex and FADS1-rs174547 genotype distribution in the subgroup of 410 individuals
22 included in the transcriptomic analysis are shown in Supplementary Table 5. In the analysis of
23 the whole transcriptome after application of statistical threshold filter for false discovery rate
24 (FDR; q -value) ≤ 0.05 , only transcripts of the FADS2 gene were differentially expressed between
25 the FADS1-rs174547 genotypes (Figure 2A). The overall FADS2 expression was higher than the
26 FADS1 expression (Figure 2B) and significantly different between the FADS1-rs174547
27 genotypes, with the TT genotype being associated with lowest FADS2 expression levels (Figure
28 2B). FADS2 expression levels were significantly higher in the CC vs. TT (FC=2.07; $p=5.86e-15$;
29 $q=7.96e-10$) and CT vs. TT (FC=1.42; $p=3.48e-11$; $q=4.73e-06$) FADS1-rs174547 genotypes,

1 respectively (Figure 2B). In contrast, none of the genotype comparisons (CC vs.TT, CT vs. TT and
2 CC vs. CT) identified any significant differences in FADS1 gene expression (Figure 2B).

3 In age- and sex- adjusted analyses, FADS2 expression was negatively associated with
4 PWV (β -0.16(-0.3; -0.02); $P=0.03$). The significance was slightly increased after adjustments for
5 BMI and SBP (β -0.19 (-0.32; -0.05); $P=0.01$; Table 4). No significant association was found for
6 FADS1 expression with PWV (Table 4). Sex-stratified analyses revealed a stronger negative
7 association between FADS 2 mRNA and PWV in males compared to females (Table 4).

8 **Discussion**

9 Three major findings supporting a role of omega-3 PUFA, FADS1 locus variant and FADS2
10 expression in arterial stiffness emerge from the present study. First, the FADS1 locus variant
11 was significantly associated with PWV. Second, differential associations between FADS1 locus
12 variant and PWV were observed depending on the omega-3 PUFA intake, with high omega-3
13 PUFA intake attenuating the FADS1 locus variant-dependent associations. Third, carriers of the
14 minor FADS1 locus variant exhibited increased expression of FADS2 without alterations of
15 FADS1 expression. Taken together, these findings point to an association of FADS with PWV
16 contingent on omega-3 PUFA intake.

17 The association of higher EPA and DHA intake with lower PWV lost statistical
18 significance after adjustments in the present study. In a subgroup analysis of the Age,
19 Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) Study, circulating levels of EPA and
20 DHA, but not FFQ-evaluated fish oil consumption were associated with lower carotid-femoral
21 PWV¹². Likewise, in 351 subjects from the HealthTrack Study, omega-3 PUFA intake was not
22 significantly associated with brachial ankle PWV¹³. Beneficial effects of omega-3 PUFA on
23 arterial stiffness have received support from interventional studies showing lowered PWV after
24 fish oil supplementation³⁰. Cardiovascular risk factors, PUFA levels and doses, as well as
25 differential effects of EPA and DHA are some possible factors behind the conflicting results from
26 both observational studies of PUFA intake and/or levels and clinical trials of omega-3 PUFA
27 supplementation³¹.

28 In addition to dietary intake, endogenous biosynthesis from plant derived shorter
29 chain omega-3 PUFA contribute to the systemic and tissue levels of EPA and DHA³². This

1 conversion of PUFA involves the desaturase activities of the FADS enzymes, which in turn are
2 partially determined by genetic variations^{15, 33}. In the present study, the minor C allele of the
3 FADS1-rs174547 genotype was associated with lower carotid-femoral PWV. These findings are
4 in line with the lower risk recently reported to be associated with this genotype for a range of
5 cardiovascular diseases^{16, 29, 34}, and extend the observation to subclinical arterial disease in
6 otherwise healthy individuals.

7 Importantly, although the interactions between genotype and fatty acid intake
8 were not significant, we observed that unlike in CC carriers, in CT and TT carriers there was a
9 significant association between higher EPA/DHA intake and lower PWV. Moreover, we
10 observed only in the group with low PUFA intake that there was an association between the
11 FADS1 locus variant and PWV (compared to CC, increasing PWV for CT and TT). This suggests
12 that high omega-3 PUFA intake may compensate for an unfavorable FADS1 locus genotype

13 Desaturase-dependent biosynthetic pathways may represent a less important source of
14 total DHA and EPA at high fish consumption³². The consequences of this for the cardiovascular
15 phenotype, as suggested by the present results for carotid femoral PWV, have however not
16 previously been reported. Taken together, these findings raise the notion that an unfavorable
17 FADS1 locus variant effect on PWV could be neutralized by high omega-3 PUFA intake, and that
18 less pronounced beneficial effects of omega-3 PUFA intake could be anticipated in carriers of
19 the protective FADS1 locus variant.

20 An increase in the dietary omega-6 to omega-3 ratio has previously been associated
21 with increased risk of acute coronary syndromes³⁵. Likewise, the ratio of the downstream lipid
22 mediators from these PUFAs, *e.g.* resolvin D1 to leukotriene B4 ratio, has been associated with
23 preclinical atherosclerosis³⁶. However, results are conflicting on how alterations in PUFA status
24 as a result of FADS1 polymorphisms are modified by dietary intake of EPA and DHA. The large
25 observational CHARGE consortium reported that association of FADS1 variants with circulating
26 EPA and DHA were independent of fish consumption¹⁵, but that this association may vary
27 depending on the blood fraction used for PUFA measurements, *e.g.* plasma vs. erythrocyte
28 membranes³⁷.

1 In addition to reducing Δ 5-desaturase activity and lowering AA levels¹⁵, the rs174547 CC
2 genotype increases FADS2 (but not FADS1) mRNA expression and Δ 6-desaturase activity,
3 resulting in increased levels of DHA in aortic valves²⁹. Although the FADS1 locus variant has
4 been associated with altered FADS1 expression in other tissues, the results of the
5 transcriptomic analysis in the present study supports FADS2 (but not FADS1) expression being
6 increased in the blood from carriers of the minor FADS1 locus variant. Importantly, FADS2
7 expression was inversely associated with PWV, reinforcing a connection between FADS1 locus
8 variant and arterial stiffness through FADS2 expression.

9 Several differential observations were noted between males and females in the present
10 study. Consistent with other studies³⁸, males had higher total PUFA intake whereas the PUFA
11 proportion of total energy intake was higher in females. In addition, men had higher omega-6
12 to omega-3 ratio compared with the intake in females. Also, the negative association between
13 EPA and DHA intake and PWV was significant for males but not for females in the age-adjusted
14 analysis, albeit non-significant in both sexes for the fully adjusted models. Furthermore, the
15 FADS genotype and FADS2 expression predicted PWV significantly only in males but not in
16 females, which is similar to the previously reported association between the consumed and
17 circulating proportions of omega-3 PUFAs being less pronounced in men who are homozygous
18 for the major allele of another FADS1 SNP (rs174550 in linkage disequilibrium with rs 174547)³⁹.
19 Other possible factors underlying the sex-specific associations between PUFA intake and
20 cardiovascular phenotypes in the present and previous⁴⁰ studies include differences in risk
21 factors, and comorbidities, and metabolic differences across the sexes. The latter has received
22 support from the observation that females have a significantly greater increase in EPA content
23 of plasma phospholipids compared to males over the same period on the same diet⁴¹.

24 The possible mechanisms involved in the role of EPA and DHA intake with reduced
25 arterial stiffness may involve changes in the downstream lipid mediators. Indeed, whereas
26 omega-6-derived leukotriene B4 has been associated with increased PWV⁴², proresolving
27 mediators derived from DHA and EPA exert beneficial effects on the vascular wall^{3,4}.

28 The present study is the first to link FADS1 rs174547 genotype with dietary omega-3
29 PUFA intake to a beneficial arterial phenotype in the absence of known cardiovascular disease.

1 The strengths include analyses based on a large general and initially healthy population-based
2 cohort with the availability of detailed information on diet, genetics and extensive
3 cardiovascular phenotyping, including PWV. Certain limitations should however be
4 acknowledged. We did not measure the levels of EPA and DHA in this study. Ideally those
5 measures should be performed in erythrocyte membranes, which are not available in the
6 STANISLAS family cohort. Even if diet was assessed through declarative data over the past 3
7 months and can underestimate the real consumption, FFQ is well detailed for PUFA intake and
8 a reliable tool to evaluate the habits of food consumption regarding PUFAs²². Nonetheless, we
9 did not collect information on dietary supplements rich in n-3 PUFA, which is used by 13.8% of
10 French adults⁴³. In addition, we did not measure downstream PUFA-derived lipid mediators.
11 The observational and cross-sectional design of the study also means that no causal relation
12 can be concluded for the observed associations with arterial stiffness.

13 In summary, the present study identified significant associations between omega-3
14 PUFA intake, FADS1 locus variant, FADS2 expression, and carotid-femoral PWV. Importantly,
15 the unfavorable genotype-effect on arterial stiffness was blunted by a high omega-3 PUFA
16 intake. In conclusion, the FADS1 locus variant may affect the impact of omega-3 PUFA intake on
17 subclinical cardiovascular disease. These findings may have implications for identifying
18 responders and non-responders to omega-3 supplementation and open up for precision
19 medicine through personalized dietary counselling in cardiovascular prevention.

20

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33

34 **Disclosures**

35 None

36

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7

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1 **Table 1:** Study Population Characteristics

	All	Men	Women	p-value
N	1464	711	753	
Socio-demographics				
Age (years)	55(34-60)	57 (34-61)	54 (34-59)	<0.001
Smokers	296 (20%)	152 (21%)	144 (19%)	0.28
Education level				0.58
Low	540 (37%)	272 (38%)	268 (36%)	
Intermediate	224 (15%)	107 (15%)	117 (16%)	
High	699 (48%)	332 (47%)	367 (49%)	
BMI (kg/m ²)	25(22.5-28.3)	25.8 (23.7-28.7)	24.0 (21.7-27.8)	<0.001
Energy expenditure (MET-minute/week)	1782(656-4158)	2322 (792-5976)	1440 (554-3192)	<0.001
Food intake				
Energy intake (kcal/day)	2242(1764-2865)	2545 (2061-3077)	1951 (1538-2498)	<0.001
Carbohydrates (g/day)	254.9±99.9	286.1 ± 102.5	225.5 ±87.7	<0.001
Lipids (g/day)	97.8±40.5	106.0 ± 39.5	90.0 ±39.9	<0.001
Proteins (g/day)	99.5±36.7	109.8 ± 37.0	89.7 ± 33.6	<0.001
Saturated fat (g/day)	37.6±16.2	40.9 ± 16.0	34.3 ± 15.8	<0.001
MUFA (g/day)	38.8±17.3	42.1 ± 17.1	35.6 ± 16.9	<0.001
PUFA (g/day)	14.7±7.7	15.6 ± 7.3	13.9 ± 8.0	<0.001
EPA (mg/day)	158.1 ± 125.1	165.6 ± 119.2	151.0 ±130.1	0.03
DHA (mg/day)	239.2 ± 187.7	250.5 ± 177.7	228.5 ± 192.5	0.02
Ratio w6/w3	7.3 ± 2.6	7.5 ± 2.7	7.1 ± 2.6	0.002
Carbohydrates (mg/kcal/day)	107.7 ± 20.5	108.0 ± 20.4	107.5 ± 20.5	0.63
Lipids (mg/ kcal/day)	41.1 ± 8.0	39.9 ± 7.7	42.2 ± 8.0	0.001
Proteins (mg/ kcal/day)	42.4 ± 8.1	41.8 ± 7.7	42.9 ± 8.5	0.008
Saturated fat (mg/ kcal/day)	15.8 ± 3.6	15.4 ± 3.5	16.1 ± 3.6	0.001
MUFA (mg/ kcal/day)	16.3 ± 4.1	15.9 ± 3.9	16.7 ± 4.2	0.001
PUFA (mg/ kcal/day)	6.2 ± 2.2	5.9 ± 1.9	6.5 ± 2.4	0.001
EPA (µg/ kcal/day)	69.6 ± 53.5	65.2 ± 47.5	73.8 ± 58.4	0.002
DHA (µg/ kcal/day)	105.4 ± 79.1	98.6 ± 70.7	111.8 ± 85.9	0.001
Clinics				
Metabolic syndrome	332 (27%)	180 (31%)	152 (24%)	<0.001
Diabetes	76 (5%)	49 (7%)	27 (4%)	0.004
Fasting glucose (g/L)	0.9(0.8-1)	0.9 (0.9-1.0)	0.9 (0.8-0.9)	<0.001
HbA1C (%)	5.6(5.3-5.8)	5.6 (5.4-5.8)	5.5 (5.3-5.8)	0.10
Use of anti-diabetic drugs	58 (4%)	39 (6%)	19 (3%)	0.004
Elevated triglycerides	385 (26%)	234 (33%)	151 (20%)	<0.001
Triglycerides (g/L)	0.9(0.7-1.3)	1.0 (0.7-1.4)	0.9 (0.8-0.9)	<0.001
Total cholesterol (g/L)	2.1(1.9-2.4)	2.1 (1.8-2.4)	2.1 (1.9-2.5)	<0.001
HDL-C (g/L)	0.6(0.5-0.7)	0.5 (0.4-0.6)	0.6 (0.5-0.7)	<0.001
LDL-C (g/L)	1.3(1.1-1.6)	1.3 (1.1-1.6)	1.3 (1.1-1.6)	0.21
Use of lipid-lowering drugs	208 (14%)	127 (18%)	81 (11%)	<0.001
Hypertension	565 (39%)	326 (49%)	239 (34%)	<0.001

24h-SBP (mmHg)	120.1 ± 10.2	123.6 ± 9.3	117.0 ± 10.0	<0.001
24h-DBP (mmHg)	74.3 ± 7.2	76.2 ± 6.9	72.6 ± 7.0	<0.001
Use of anti-hypertensive drugs	277 (19%)	151 (21%)	126 (17%)	0.03
FADS1 rs174547 genotype	CC: 134 (9%) CT: 663 (45%) TT: 667 (46%)	CC: 62 (9%) CT: 329 (46%) TT: 320 (45%)	CC: 72 (10%) CT: 334 (44%) TT: 347 (46%)	0.71

1 BMI: body mass index, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids,
2 EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, HbA1C:
3 glycated haemoglobin, HDL-C: plasma high density cholesterol, LDL-C: plasma low density
4 cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure. Hypertension is defined
5 as elevated blood pressure (130/80) and/or declared hypertension and/or use of at least one
6 anti-hypertensive drug, diabetes is defined by high fasting glucose (>1.26g/L) and/or declared
7 diabetes and/or use of at least one anti-diabetic drug. Metabolic Syndrome definition is
8 specified in the Methods section.

13 **Table 2.** Multivariate association between the FADS1 rs174547 genotype (risk per T allele) and
14 pulse wave velocity (N=1464)

rs174547	β (95% CI)	p-value
M0	0.15 (0.04 ; 0.27)	0.007
M1	0.15 (0.04 ; 0.27)	0.01
M1 + EPA	0.15 (0.04 ; 0.26)	0.01
M1 + DHA	0.15 (0.03 ; 0.26)	0.01
M1 + total energy intake	0.15 (0.04 ; 0.27)	0.01

15 *Mixed model with random effect on family*

16 *M0: adjusted on age and sex*

17 *M1: M0 + adjusted for BMI and SBP*

1 **Table 3.** Association between FADS1 rs174547 genotype (risk per T allele) and PWV stratified by
 2 PUFAs median (</≥)

	< Median		≥ Median	
	β (95% CI)	p-value	β (95% CI)	p-value
EPA				
<i>M0</i>	0.17 (0.02 ; 0.33)	0.03	0.13 (-0.03 ; 0.29)	0.11
<i>M1</i>	0.21 (0.05;0.37)	0.01	0.09 (-0.07;0.26)	0.26
DHA				
<i>M0</i>	0.20 (0.04;0.36)	0.01	0.11 (-0.05;0.27)	0.18
<i>M1</i>	0.23 (0.07;0.39)	0.006	0.07 (-0.09;0.24)	0.37
<i>Ratio w6/w3</i>				
<i>M0</i>	0.20 (0.04;0.36)	0.02	0.09 (-0.06 ; 0.25)	0.24
<i>M1</i>	0.18 (0.02 ; 0.34)	0.02	0.10 (-0.07 : 0.26)	0.25

3 *Mixed model with random effect on family*

4 *M0: adjusted on age and sex*

5 *M1: M0 + adjusted on bmi, and SBP*

6 *The medians were 0.13 g/day for EPA, 0.19 g/day for DHA, 6.87 for ratio w6/w3*

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1 **Table 4. Associations between FADS1 and FADS2 gene expression and Pulse Wave Velocity in**
 2 **the subgroup of STANISLAS cohort with transcriptomic data (n=410).**

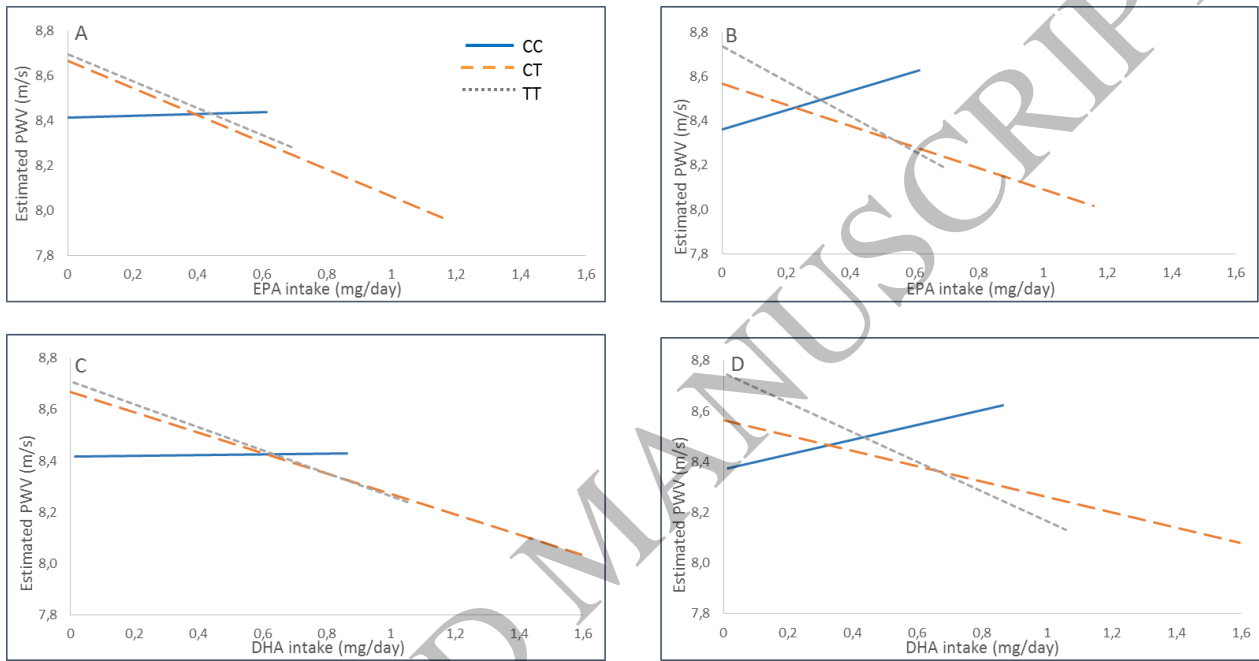
	β (95% CI)	p-value
All (n=410)		
FADS1		
M0	-0.04 (-0.26 ; 0.18)	0.73
M1	-0.10(-0.34 ;0.14)	0.40
FADS2		
M0	-0.16(-0.30 ; -0.02)	0.03
M1	-0.19(-0.33 ; -0.05)	0.01
Men (n=205)		
FADS1		
M0	-0.09 (-0.4 ; 0.22)	0.57
M1	-0.07 (-0.42 ; 0.28)	0.71
FADS2		
M0	-0.27 (-0.48 ; -0.05)	0.01
M1	-0.30 (-0.70 ; -0.08)	0.02
Women (n=205)		
FADS1		
M0	-0.01 (-0.16 ;0.28)	0.97
M1	-0.16 (-0.47 ; 0.15)	0.32
FADS2		
M0	-0.07 (-0.26 ; 0.13)	0.48

3 *M0: adjusted on sex and age*

4 *M1: M0 + adjusted on BMI and SBP*

5

1 **Fig 1.** Association of PUFA intake from either EPA (Panels A and B) or DHA (Panels C and D)
 2 with Pulse Wave Velocity (PWV) stratified by different genotypes of *FADS1* rs174547 after
 3 estimation with linear regression adjusted on sex and age (Model 0; Panels) and on sex, age,
 4 BMI and SBP (Model 1). Solid blue lines represent homozygous for minor allele (CC), dotted
 5 orange lines represent heterozygous (CT) and scattered grey lines represent homozygous for
 6 major allele (TT). (all interactions are non-significant – N=1464)



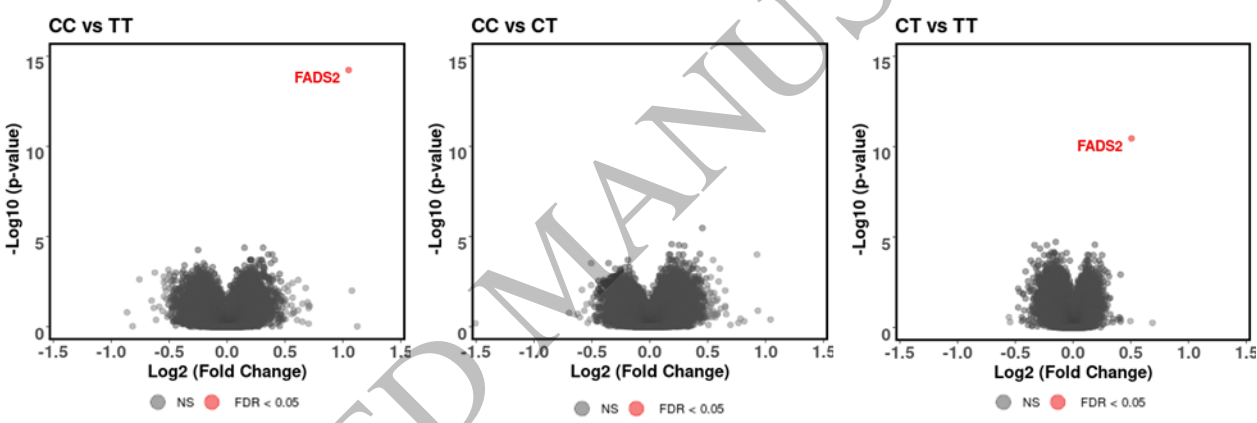
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2 **Figure 2.** Association of FADS gene expression with FADS1-rs174547 genotype in a subgroup of
3 410 individuals. A. Volcano plots representing the significance (-Log₁₀(p-value)) vs. the fold
4 change (Log₂(Fold Change)) for differentially expressed genes in CC vs.TT (left), CC vs. CT
5 (middle) and CT vs. TT (right) comparisons. Each gene is represented by a dot with gray dot
6 corresponding to FDR>0.05 and red dot to FDR<0.05. C. Normalized Intensities of FADS2 (left
7 panel) and FADS1 (right panel) according to FADS1-rs174547 genotype. Normalized intensities
8 have been extracted form cluster IDs TC1100011050.hg.1 and TC1100013022.hg.1,
9 representing FADS1 and FADS2 gene expression respectively. Mann-Whitney Wilcoxon
10 significant at: *p<0.05, **p<0.01, *** p<0001. ns: non-significant; p>0.05.

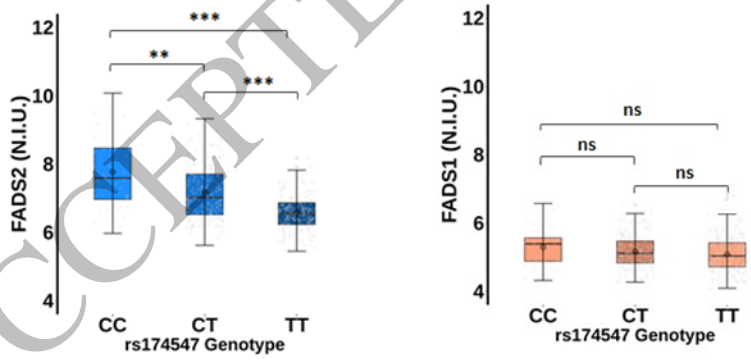
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A.



17

B.



1 Patrick Rossignol, MD, PhD, is a Nephrologist and Vascular medicine specialist, ESC/ESH certified
2 hypertension specialist, Professor of Therapeutics at the University of Lorraine, France.
3 He is heading the Nancy University Hospital Inserm Plurithematic Clinical Investigation Centre.
4 He is coordinating the French multidisciplinary network F-CRIN (French Clinical research Infrastructure
5 Network): Cardiovascular and Renal Clinical Trialists (INI-CRCT) www.inicrct.org.
6 He is mainly involved in clinical trials, in heart failure, hypertension, and chronic kidney disease.
7 Hyperkalemia is one of his main areas of interest (<https://expertscape.com/ex/hyperkalemia>).
8 He has published more than 400 peer review publications and is the co-founder of the SME CardioRenal.

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