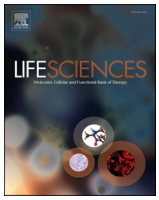
Life Sciences 351 (2024) 122796

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie



The serum metabolomic profiles of atrial fibrillation patients treated with direct oral anticoagulants or vitamin K antagonists

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ARTICLE INFO

Keywords:
Metabolomics
Lipoproteins
NMR
Anticoagulation
Atrial fibrillation

ABSTRACT

Aims: Long-term oral anticoagulation is the primary therapy for preventing ischemic stroke in patients with atrial fibrillation (AF). Different types of oral anticoagulant drugs can have specific effects on the metabolism of patients. Here we characterize, for the first time, the serum metabolomic and lipoproteomic profiles of AF patients treated with anticoagulants: vitamin K antagonists (VKAs) or direct oral anticoagulants (DOACs).

Materials and methods: Serum samples of 167 AF patients (median age 78 years, 62 % males, 70 % on DOACs treatment) were analyzed via high resolution ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Data on 25 metabolites and 112 lipoprotein-related fractions were quantified and analyzed with multivariate and univariate statistical approaches.

Key findings: Our data provide evidence that patients treated with VKAs and DOACs present significant differences in their profiles: lower levels of alanine and lactate (odds ratio: 1.72 and 1.84), free cholesterol VLDL-4 subfraction (OR: 1.75), triglycerides LDL-1 subfraction (OR: 1.80) and 4 IDL cholesterol fractions (ORs ~ 1.80), as well as higher levels of HDL cholesterol (OR: 0.48), apolipoprotein A1 (OR: 0.42) and 7 HDL cholesterol fractions/subfractions (ORs: 0.40–0.51) are characteristic of serum profile of patients on DOACs' therapy.

Significance: Our results support the usefulness of NMR-based metabolomics for the description of the effects of oral anticoagulants on AF patient circulating metabolites and lipoproteins. The higher serum levels of HDL cholesterol observed in patients on DOACs could contribute to explaining their reduced cardiovascular risk, suggesting the need of further studies in this direction to fully understand possible clinical implications.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia with clinical relevance observed in medical practice [1]. Long-term oral anticoagulation is the mainstay therapy for ischemic stroke prevention in AF patients [2]. For many years, the therapeutic standard for oral anticoagulation were dose-adjusted vitamin K antagonists (VKAs). Direct-acting oral anticoagulants (DOACs) have extended the armamentarium of clinicians in AF thromboprophylaxis. DOACs have shown similar efficacy and safety profiles as compared with VKAs but at fixed dosages, removing the inconvenience of dose adjustment necessary by VKAs. DOACs have steadily gained ground and now are the preferred anticoagulants in the clinical practice [3].

Many pharmacological agents exert pleiotropic effects that can

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https://doi.org/10.1016/j.lfs.2024.122796
Received 16 February 2024; Received in revised form 3 May 2024; Accepted 4 June 2024
Available online 7 June 2024

provide additional benefits or increase harm. The antithrombotic activity of oral anticoagulants has been extensively studied and described, conversely their pleiotropic effects still need to be characterized in order to elucidate their underlying molecular mechanisms and clinical implications [4]. Omics sciences may represent the perfect instrument to meet this goal. In particular, metabolomics provides the analysis of subjects and their biological characteristics within the dynamic context of a disease process, thus it enables the molecular characterization of a disease and it can provides insight into individual susceptibility to drug administration [5–9].

Here we present, for the first time to best of our knowledge, an extensive characterization of the serum metabolomic and lipoproteomic profiles of AF patients treated with different oral anticoagulation therapies (DOACs and VKAs) and enrolled in the framework of the Strat-AF study [10].

2. Material and methods

2.1. Study population

This pilot observational monocentric study comprised a population of 177 elderly patients diagnosed with atrial fibrillation (AF). These patients are a subset of those enrolled in the framework of the Stratification of cerebral bleeding risk in AF (Strat-AF) study from the outpatient clinic of Thrombosis Center of the Careggi University Hospital in Florence, where they are monitored for the management of oral anticoagulation therapy in primary or secondary prevention of thromboembolic events. The Strat-AF (http://www.strat-af.it) is a single-center, observational, prospective study aimed at evaluating if biological markers, either clinical, circulating and neuroimaging-based can improve the prediction of bleeding risk in AF patients under treatment with any type of oral anticoagulants. Details on the Strat-AF study design, inclusion/exclusion criteria, and methodology can be retrieved from previous publications [10,11]. Before the DOACs therapy, 49 patients had been treated extensively with VKAs. To avoid biases induced by the change of therapy, only patients who had been under DOACs treatment for at least 12 months at the time of sampling were included in this analysis. This criterion led to the exclusion of 10 patients.

According to a standard post-hoc power analysis [12], using a *t*-test of means as the test statistics, and fixing an alpha level of 0.05 for a significant comparison, 115 patients treated with DOACs and 52 patients treated with VKAs demonstrated to be enough to detect small-medium effects (Cohen's d ~ 0.47) with a statistical power of 80 %.

2.2. Ethical issue

All subjects gave their written informed consent for inclusion in the study. The study, which complies with the Declaration of Helsinki and its later amendments, was approved in March 2017 by the Ethics Committee of the Careggi University Hospital (Project identification code 16RFAP).

2.3. Sample collection and NMR analysis

After fasting overnight, serum samples were collected, following the standard operating procedures for metabolomics [13]. Then samples were immediately stored at −80 °C pending NMR analysis.

All NMR spectra were acquired using a Bruker 600 MHz spectrometer (Bruker BioSpin) operating at 600.13 MHz proton Larmor frequency provided with an automatic and refrigerated (6 °C) sample changer (SampleJet™, Bruker BioSpin), and a BTO 2000 thermocouple utilized for temperature stabilization (~0.1 K at the sample). All samples were acquired at 310 K. A standard nuclear Overhauser effect spectroscopy pulse sequence NOESY 1Dpresat was applied to detect signals of low and high-molecular weight molecules present in each sample in concentrations above the NMR detection limit. To ensure high spectral quality and reproducibility, the spectrometer was calibrated daily following strict standard operating procedures. A detailed description of sample preparation procedures, instrument configuration and NMR parameters' setting, acquisition and processing can be retrieved from previous publications [14,15].

A panel of 41 metabolites were unambiguously identified and quantified using Bruker IVDr Quantification in Plasma/Serum B.I. Quant-PS platform™ (version 2.0.0). Covariates with >20 % of observation under the LOQ (limit of quantification) were excluded from the present analysis, thus the system was reduced to 25 metabolites including: amino acids and their derivatives (alanine, creatine, creatinine, glutamine, glycine, histidine, isoleucine, leucine, methionine, *N*,*N*-Dimethylglycine, phenylalanine, tyrosine, valine), carboxylic acids (acetate, citriate, formate, lactate, succinate), keto acids and derivatives (3-hydroxybutyrate, acetoacetate, acetone, pyruvate), and other compounds such as glucose, dimethylsulfone, and trimethylamine-N-oxide.

The identification and quantification of 112 lipoprotein-related parameters was performed utilizing the Bruker IVDr Lipoprotein Subclass Analysis platform™ (version 1.0.0). This platform provides information about the content of triglycerides, cholesterol, free cholesterol, phospholipids, Apo-A1, Apo-A2 and Apo-B100 of the main fractions and subfractions of VLDL, IDL, LDL and HDL classes.

2.4. Statistical analysis

All data analyses were performed in the "R" statistical environment (version 4.3.2).

The Random Forest (RF) algorithm [16] was used for classification of the groups of interest (patients treated with DOACs vs. VKAs) on the basis of the metabolomic or lipoproteomic profile. The R package 'Random Forest' was used to grow a forest of 1000 trees, using the default settings. To reduce the potential bias due to an unbalanced number of samples per group, the function option "sampsize" was used. Accuracy, sensitivity, specificity, and the area under the receiver operating characteristic curve (AUROC) were calculated according to the standard definitions. Each of these four parameters was assessed for significance against the null hypothesis of no prediction accuracy in the data by means of a 10^2 randomized class-permutations test [17]. Before to calculate the RF model, metabolites with values lower than the LOQ were imputed to the median value. Imputed data were used only for this specific analysis.

Demographic and baseline characteristics were examined to point out differences among patients included in the two treatment groups. The Wilcoxon rank-sum test was used for numerical variables and the chi-square test for categorical variables.

The net effect of each metabolic variable (metabolites or lipoprotein-related parameters) on the treatment administered was estimated by multivariable logistic regression analysis, which includes as covariates: sex, history of heart failure and OAC treatment duration (these parameters emerged as significant from the abovementioned univariate analysis). Statistical significance for each of the variables in relation to treatment administered was calculated by means of a Wald test. *P*-values were adjusted for multiple testing using the false discovery rate (FDR) procedure with the Benjamini-Hochberg [18] correction at $\alpha = 0.05$.

3. Results

The analyses were conducted on 167 patients diagnosed with atrial fibrillation. At the moment of sample collection 115 patients (70 %) were on DOACs' treatment, and 52 patients (30 %) on VKAs. Demographic and clinical characteristics of the study population are reported in Table 1 for the entire population and stratified by the oral anticoagulant administered. Further details of pharmacological history of enrolled patients are reported in **Supplementary Table S1**.

To characterize the metabolic differences between patients treated with DOACs and VKAs, random forest (RF) models were built using

Table 1
Demographic and clinical characteristics of the entire study population and stratified according to the anticoagulant therapy administered.

	Study Population (167)	DOACs (115)	VKAs (52)	p-value adjusted for FDR
		Demographic characteristics,		
Age (yrs), median (IQR)	78.5 (74.7–82.3)	78.4 (74.6–82.3)	79.2 (74.7–82.2)	0.963
BMI (Kg/m^2), median (IQR)	26.1 (24.6–27.8)	25.9 (24.4–27.6)	26.6 (24.6–28.4)	0.414
Gender, Males, n (%)	104 (62)	63 (55)	41 (79)	0.048
Physical Activity, No, n (%)	105 (63)	71 (62)	34 (65)	0.919
Current smokers, Yes, n (%)	13 (8)	8 (7)	5 (10)	0.919
Ex-smokers, Yes, n (%)	94 (56)	63 (55)	31 (60)	0.919
Alcohol consumption, Yes, n(%)	87 (52)	54 (47)	33 (63)	0.299
		Medical history, Yes, n (%)		
Stroke	38 (23)	27 (23)	11 (21)	0.963
Ischemic Heart Disease	17 (10)	9 (8)	8 (15)	0.567
Chronic Heart Failure	22 (13)	8 (7)	14 (27)	0.014
Diabetes	23 (14)	16 (14)	7 (13)	1
Hypertension	135 (81)	95 (83)	40 (77)	0.826
Dyslipidemia	82 (49)	52 (45)	30 (58)	0.517
Renal Disease	10 (6)	5 (4)	5 (10)	0.66
Mild Cognitive Impairment	76 (47)	55 (50)	21 (40)	0.66
Pharmacological Therapy				
Lipid-Lowering Medications, Yes (%)	64 (38)	41 (36)	23 (44)	0.66
Lipid-Lowering Medications, n	Atorvastatin: 30 Pravastatin: 5 Rosuvastatin: 3 Simvastatin: 22 Other: 4	Atorvastatin: 18 Pravastatin: 4 Rosuvastatin: 3 Simvastatin: 15 Other: 1	Atorvastatin: 12 Pravastatin: 1 Rosuvastatin: 0 Simvastatin: 7 Other: 3	/
Statin Intensity*, n	Low: 2 Moderate: 36 High: 5	Low: 2 Moderate: 21 High: 4	Low: 0 Moderate: 15 High: 1	/
Oral Anticoagulation Drugs, n (%)	/	Apixaban: 38 (33) Dabigatran: 41 (36) Edoxaban: 13 (11) Rivaroxaban: 23 (20)	Warfarin: 49 (94) Acenocoumarol: 3 (6)	/
OAC Treatment Duration (months), median (IQR)	29 (18–47.7)	26 (16–38)	63 (18–90)	0.0002
		Clinical Information, median (IQR)		
Systolic Blood Pressure (mm Hg)	130 (125–140)	130 (125–140)	130 (125–140)	0.919
Diastolic Blood Pressure (mm Hg)	80 (70–80)	80 (70–80)	80 (70–80)	0.919
Ejection Fraction (%)	60 (57–63)	60 (58–63)	60 (57–62.7)	0.826
White Blood Cells (·10^9 L)	7 (6.2–7.5)	7 (6.2–7.5)	7.1 (6.2–7.4)	1
Red Blood Cells (·10^{12} L)	4.7 (4.5–4.8)	4.7 (4.5–4.8)	4.6 (4.5–4.8)	0.660
Hemoglobin (g/dL)	13.9 (13.2–14.5)	14 (13.2–14.4)	13.9 (13.2–14.8)	0.660
Hematocrit (%)	42.5 (40.9–44.3)	42.3 (40.5–43.8)	42.9 (40.9–45.6)	0.306
Platelet Count (·10^9 L)	205 (183–228.3)	212 (189–233.7)	184 (183–204.7)	0.234
MOCA	21.9 (20.4–23.5)	21.4 (20–23.2)	22.5 (20.4–24.2)	0.299
HAS-BLED	2 (1–2)	1 (1–2)	2 (1–2)	0.074
CHA_2DS_2-VASC	4 (3–4)	4 (3–4)	4 (3–4)	0.919

Physical Activity: defined as at least 3 days/week of moderate physical activity for at least 30 min; MOCA: MOntreal Cognitive Assessment; HAS-BLED: acronym for Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly, Drugs/alcohol concomitantly; CHA_2DS_2-VASC: Congestive Heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke, Vascular disease, Age 64–74 years, Sex category.
* Statin intensity according to ACC/AHA 2018 Guideline on the Management of Blood Cholesterol.

separately metabolites and lipoprotein-related parameters. The RF model built on metabolite concentrations shows a significant differential clustering between the two groups of interest: 0.73 AUROC (p-value = 0.01), 70.0 % accuracy (p-value = 0.01), 72.2 % sensitivity (p-value = 0.01), and 65.4 % specificity (p-value = 0.01) (Fig. 1A).

The RF model built on the lipoproteomic profile did not show any significant discrimination between patients treated with DOACs and VKAs: 0.59 AUROC (p-value >0.05), 55.7 % accuracy (p-value >0.05), 55.7 % sensitivity (p-value >0.05), and 55.8 % specificity (p-value >0.05) (Fig. 1B).

Concentrations of each metabolite, and lipoprotein-related parameter (fractions and sub-fractions) were used to build logistic regression models for the evaluation of their net effect on patients treated with DOACs and VKAs. These multivariable models were adjusted for sex, history of heart failure and OAC treatment duration. Complete results are reported in Table 2. Higher levels of alanine and lactate (both not significant after FDR correction), free cholesterol VLDL-4 subfraction, triglycerides LDL-1 subfraction and 4 IDL fractions show to be associated with the treatment with VKAs, whereas higher levels of HDL cholesterol, apolipoprotein A1 and 7 HDL cholesterol fractions and subfractions are characteristic of patients in treatment with DOACs. Moreover, several VLDL cholesterol and triglycerides subfractions showed near-significant (p-value <0.1 after FDR correction) higher levels in VKAs patients.

4. Discussion

In this study we have investigated the levels of serum metabolites and lipoproteins via ^{1}H NMR spectroscopy in AF patients treated with different anticoagulation drugs: DOACs and VKAs. We have examined a cohort of 167 elderly patients diagnosed with atrial fibrillation in the framework of the Strat-AF study, among them 115 patients were in treatment with DOACs, and 52 patients were in treatment with VKAs.

Patients treated with DOACs and VKAs show significantly different metabolite profiles (discrimination accuracy of the RF model 70.0 %, p-value 0.01), and from the logistic regression analysis emerged that lactate and alanine present significantly (before FDR correction) higher levels in patients treated with VKAs. Alanine and lactate are two relevant precursors of gluconeogenesis in the liver, along with glutamine

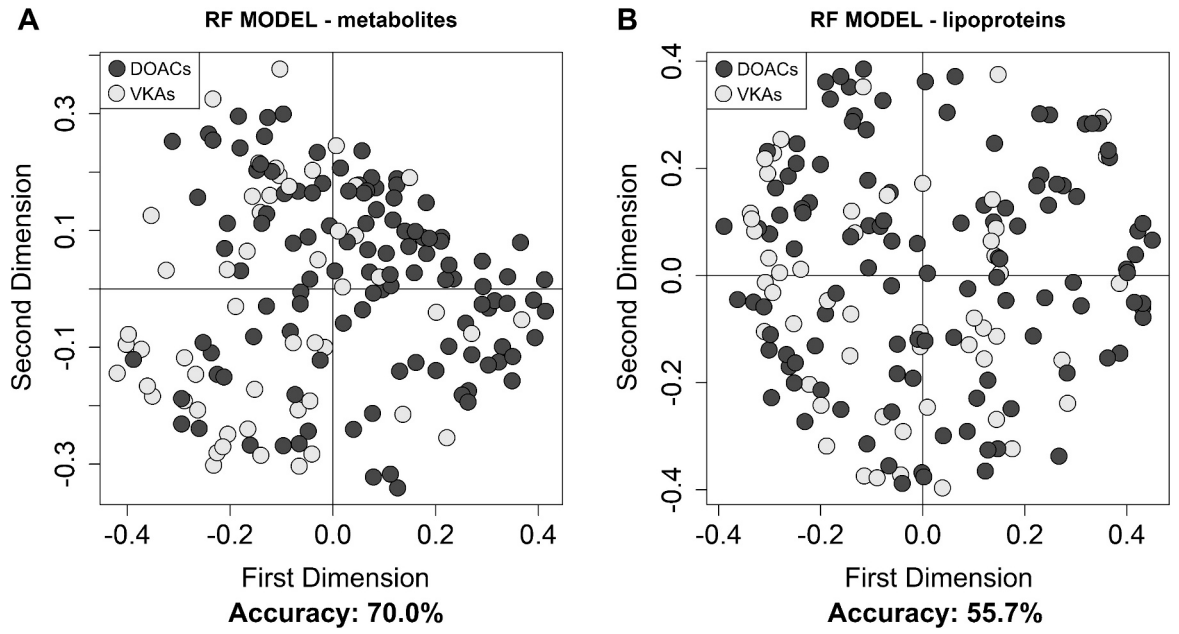

Fig. 1. Metabolomic/lipoproteomic discrimination between AF patients treated with DOACs and VKAs. Proximity plots of the RF model discriminating AF patients treated with DOACs (black dots) and treated with VKAs (light grey dots) using A) the 25 metabolites; B) the 112 lipoprotein-related parameters quantified.

gluconeogenesis which is predominantly in the kidney [19]. This evidence could explain why metabolites involved in the energetic metabolism but in different body districts show opposite trends in serum profiles of patients treated with DOACs and with VKAs. Furthermore, it is known that compared to warfarin, DOACs are less reliant on hepatic clearance but have an impact on the hepatorenal body clearance [20]. However, in our population patients treated with DOACs as compared to VKAs showed lower (but not statistically significant) levels of serum creatinine [21].

The logistic regression analysis clearly pointed out that patients on DOACs' treatment are characterized by significantly higher levels of HDL-cholesterol and Apo-1 (fractions and subtractions), and reduced levels of IDL cholesterol, VDL cholesterol and triglycerides. To the best of our knowledge, this evidence is reported here for the first time; therefore, further studies, involving a larger number of participants and conducted across multiple centers, need to be performed to characterize the biological mechanisms that underlie the different lipoprotein metabolism in these patients. However, we could hypothesize a pleiotropic effect of DOACs, distinct from the initial indications of these drugs. HDL-cholesterol has been considered inversely associated with cardiovascular mortality and stroke for many years [22–25]. On the contrary, IDL cholesterol is reported to be a significant risk marker for coronary heart disease [26,27]. Recent studies have demonstrated that patients treated with standard-dose DOACs have a lower risk of all-cause death, cardiovascular death, and any stroke compared to those treated with warfarin [28], however, this evidence should be further investigated. The association between DOACs and lower total mortality is not fully explained by the cardiovascular death or ischemic events, so that it represents evidence till now unexplained. The higher levels of HDL-cholesterol and the lower levels of IDL-cholesterol (as well as VLDL cholesterol and triglycerides although only near significant) observed in this study in patients treated with DOACs may potentially contribute to propose a protective effect of these drugs.

This investigation suggests different metabolomic and lipoproteomic alterations in serum samples of AF patients treated with different anticoagulation therapies. Although our study is innovative, some limitations should also be mentioned. Firstly, the study design did not involve the collection of samples before and after the initiation of OAC. This approach could have facilitated a more comprehensive and definitive assessment of causality between treatment types and observed differences in patient outcomes. Secondly, the lack of an independent cohort for validation and the small sample size (which prevented us from performing sex-based analyses) represent two other limitations of the present pilot study.

5. Conclusion

In conclusion, this pilot observational monocentric study has provided compelling evidence that patients with atrial fibrillation who receive different anticoagulation drugs, namely VKAs and DOACs, exhibit distinct metabolomic and lipoproteomic profiles. Our results for the first time pave the way towards understanding the molecular-level effects of anticoagulant medications on circulating metabolites and lipoproteins. Moreover, these results suggest a potential pleiotropic effect of DOACs. Consequently, further investigations in this direction are warranted to fully elucidate the clinical implications and potential protective effects of DOACs administration in atrial fibrillation patients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2024.122796.

CRediT authorship contribution statement

Alessia Vignoli: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Anna Maria Gori:** Investigation. **Martina Berteotti:** Investigation. **Betti Giusti:** Investigation. **Alessia Bertelli:** Investigation. **Ada Kura:** Investigation. **Elena Sticchi:** Investigation. **Emilia Salvadori:** Data curation. **Carmen Barbato:** Investigation. **Benedetta Formelli:** Investigation. **Francesca Pescini:** Investigation. **Rossella Marcucci:** Writing – review & editing, Supervision, Conceptualization. **Leonardo Tenori:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **Anna Poggesi:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Table 2
Association between metabolites/lipoprotein-related parameters and oral anticoagulation treatments. The logistic models are adjusted for sex, history of heart failure and OAC treatment duration. The DOACs treatment group is the reference group, thus OR > 1 means higher level in patients treated with VKAs.

	Median DOACs	Median VKAs	Odds Ratio	*p*-value	p-value adjusted for FDR
Metabolites (mmol/L)					
Trimethylamine-N-oxide	0.021	0.026	1.12	0.581	0.854
Alanine	0.452	0.518	1.72	0.008	0.102
Creatine	0.008	0.006	1.03	0.906	0.912
Creatinine	0.098	0.106	1.4	0.15	0.416
Glutamine	0.672	0.625	0.67	0.057	0.233
Glycine	0.276	0.3	1.57	0.026	0.213
Histidine	0.081	0.082	0.96	0.893	0.912
Isoleucine	0.059	0.069	1.51	0.039	0.233
Leucine	0.106	0.108	1.27	0.254	0.53
Methionine	0.056	0.056	0.76	0.213	0.53
N,*N*-Dimethylglycine	0.007	0.007	1.44	0.09	0.281
Phenylalanine	0.08	0.08	1.13	0.535	0.837
Tyrosine	0.065	0.066	1.06	0.773	0.912
Valine	0.245	0.254	1.48	0.065	0.233
Acetate	0.044	0.046	1.08	0.695	0.912
Citrate	0.166	0.171	1.18	0.436	0.778
Formate	0.042	0.039	0.65	0.047	0.233
Lactate	2.034	2.602	1.84	0.006	0.102
Succinate	0.004	0.005	0.93	0.702	0.912
3-Hydroxybutyrate	0.078	0.077	0.84	0.467	0.779
Acetoacetate	0.006	0.004	0.67	0.252	0.53
Acetone	0.059	0.058	0.84	0.408	0.778
Pyruvate	0.068	0.081	0.97	0.894	0.912
Glucose	5.286	5.511	0.98	0.906	0.912
Dimethylsulfone	0.013	0.02	0.97	0.912	0.912
Lipoprotein-related main parameters (mg/dL)					
TG	123.98	142.65	1.6	0.019	0.073
Chol	185.86	178.165	0.9	0.605	0.664
VLDL-Chol	20.74	24.03	1.62	0.018	0.073
IDL-Chol	12.83	15.675	1.85	0.003	0.042
LDL-Chol	98.37	94.28	0.84	0.394	0.515
HDL-Chol	51.42	45.25	0.48	0.005	0.046
Apo-A1	143.64	133.795	0.42	0.001	0.042
Apo-A2	28.83	27.905	0.69	0.113	0.188
Apo-B100	85.98	83.315	1.07	0.723	0.772
Lipoprotein ratios					
LDL-Chol/HDL-Chol	2.01	2.055	1.36	0.13	0.209
Apo-B100/Apo-A1	0.6	0.635	1.68	0.017	0.073
Lipoprotein fractions and sub-fractions (mg/dL)					
Triglycerides, VLDL	76.65	86.495	1.56	0.029	0.087
Triglycerides, IDL	9.37	11.945	1.52	0.036	0.097
Triglycerides, LDL	24.01	23.32	1.5	0.058	0.127
Triglycerides, HDL	12.93	12.53	1.14	0.507	0.608
Free Cholesterol, VLDL	9.1	10.085	1.51	0.043	0.102
Free Cholesterol, IDL	3.66	4.285	1.86	0.003	0.042
Free Cholesterol, LDL	32.29	30.985	0.83	0.379	0.511
Free Cholesterol, HDL	13.95	12.425	0.45	0.002	0.042
Phospholipids, VLDL	19.46	20.25	1.46	0.068	0.137
Phospholipids, IDL	6.24	6.945	1.55	0.029	0.087
Phospholipids, LDL	57.24	54.245	0.81	0.292	0.408
Phospholipids, HDL	70.71	62.31	0.44	0.002	0.042
Apo-A1, HDL	141.55	129.69	0.41	0.001	0.042
Apo-A2, HDL	29.13	28.305	0.7	0.12	0.197
Apo-B, VLDL	9.06	9.46	1.67	0.013	0.073
Apo-B, IDL	5.62	6.2	1.82	0.004	0.042
Apo-B, LDL	66.26	64.71	0.86	0.449	0.56
Triglycerides, VLDL-1	36.7	45.415	1.6	0.019	0.073
Triglycerides, VLDL-2	11.63	12.405	1.42	0.089	0.167
Triglycerides, VLDL-3	11.14	12.51	1.47	0.061	0.127
Triglycerides, VLDL-4	9.31	9.7	1.55	0.033	0.091
Triglycerides, VLDL-5	2.73	2.69	1.21	0.36	0.492
Cholesterol, VLDL-1	6.33	8.345	1.65	0.014	0.073
Cholesterol, VLDL-2	3	3.47	1.44	0.076	0.147
Cholesterol, VLDL-3	3.91	4.605	1.52	0.042	0.102
Cholesterol, VLDL-4	5.12	5.495	1.53	0.037	0.097
Cholesterol, VLDL-5	1.02	1.105	1.14	0.523	0.61
Free Cholesterol, VLDL-1	1.86	2.81	1.54	0.033	0.091
Free Cholesterol, VLDL-2	1.43	1.68	1.62	0.019	0.073
Free Cholesterol, VLDL-3	1.65	1.97	1.66	0.013	0.073

(*continued on next page*)

Table 2 (*continued*)

	Median DOACs	Median VKAs	Odds Ratio	*p*-value	p-value adjusted for FDR
Free Cholesterol, VLDL-4	2.22	2.55	1.75	0.006	0.048
Free Cholesterol, VLDL-5	0.51	0.595	1.38	0.121	0.197
Phospholipids, VLDL-1	5.88	7.53	1.61	0.019	0.073
Phospholipids, VLDL-2	2.88	3.305	1.4	0.104	0.178
Phospholipids, VLDL-3	3.67	4.105	1.53	0.04	0.102
Phospholipids, VLDL-4	5.08	5.4	1.62	0.019	0.073
Phospholipids, VLDL-5	1.47	1.565	1.13	0.558	0.638
Triglycerides, LDL-1	7.06	6.665	1.8	0.006	0.048
Triglycerides, LDL-2	2.77	2.54	1.45	0.085	0.161
Triglycerides, LDL-3	2.68	2.465	0.93	0.744	0.786
Triglycerides, LDL-4	2.95	2.835	0.96	0.853	0.861
Triglycerides, LDL-5	3.05	2.885	0.96	0.863	0.863
Triglycerides, LDL-6	4.4	4.485	1.07	0.763	0.798
Cholesterol, LDL-1	19.18	19.845	1.23	0.317	0.438
Cholesterol, LDL-2	14.8	15.53	1.14	0.518	0.61
Cholesterol, LDL-3	14.35	14.725	0.91	0.653	0.71
Cholesterol, LDL-4	13.57	11.06	0.68	0.068	0.137
Cholesterol, LDL-5	16.27	14.29	0.64	0.045	0.104
Cholesterol, LDL-6	20.24	20.17	0.77	0.195	0.294
Free Cholesterol, LDL-1	6.52	6.865	1.17	0.47	0.572
Free Cholesterol, LDL-2	5.15	5.335	1.13	0.551	0.636
Free Cholesterol, LDL-3	5.17	5	0.84	0.401	0.516
Free Cholesterol, LDL-4	4.48	4.155	0.67	0.059	0.127
Free Cholesterol, LDL-5	4.8	4.19	0.62	0.028	0.087
Free Cholesterol, LDL-6	5.36	4.97	0.75	0.155	0.241
Phospholipids, LDL-1	11.91	12.135	1.26	0.283	0.407
Phospholipids, LDL-2	8.47	9.2	1.14	0.51	0.608
Phospholipids, LDL-3	8.61	8.32	0.89	0.565	0.639
Phospholipids, LDL-4	7.98	6.49	0.67	0.06	0.127
Phospholipids, LDL-5	9.09	8.11	0.63	0.042	0.102
Phospholipids, LDL-6	11.63	11.36	0.77	0.198	0.296
Apo-B, LDL-1	11.09	11.68	1.25	0.287	0.407
Apo-B, LDL-2	8.65	9.035	1.19	0.395	0.515
Apo-B, LDL-3	9.26	9.505	0.95	0.782	0.804
Apo-B, LDL-4	9.6	7.935	0.71	0.105	0.178
Apo-B, LDL-5	11.71	10.935	0.7	0.104	0.178
Apo-B, LDL-6	16.89	17.165	0.89	0.577	0.641
Triglycerides, HDL-1	4.99	4.61	1.05	0.823	0.838
Triglycerides, HDL-2	2.19	2.3	1.32	0.157	0.241
Triglycerides, HDL-3	2.29	2.38	1.27	0.222	0.327
Triglycerides, HDL-4	3.79	3.815	1.16	0.455	0.56
Cholesterol, HDL-1	17.14	14.66	0.62	0.047	0.108
Cholesterol, HDL-2	6.76	6.435	0.64	0.061	0.127
Cholesterol, HDL-3	8.16	7.315	0.58	0.031	0.089
Cholesterol, HDL-4	17.24	16.03	0.55	0.012	0.073
Free Cholesterol, HDL-1	5.05	4.465	0.57	0.023	0.084
Free Cholesterol, HDL-2	1.86	1.78	0.66	0.072	0.142
Free Cholesterol, HDL-3	2	1.56	0.45	0.002	0.042
Free Cholesterol, HDL-4	3.43	2.905	0.51	0.005	0.046
Phospholipids, HDL-1	22.35	18.095	0.57	0.025	0.087
Phospholipids, HDL-2	10.76	10.165	0.58	0.03	0.087
Phospholipids, HDL-3	12.61	10.955	0.52	0.014	0.073
Phospholipids, HDL-4	23.28	21.67	0.51	0.006	0.048
Apo-A1, HDL-1	29.08	24.765	0.58	0.027	0.087
Apo-A1, HDL-2	17.73	15.65	0.45	0.003	0.042
Apo-A1, HDL-3	23.22	21.385	0.53	0.018	0.073
Apo-A1, HDL-4	71.3	68.31	0.53	0.008	0.057
Apo-A2, HDL-1	2.8	2.325	0.58	0.026	0.087
Apo-A2, HDL-2	2.84	2.62	0.69	0.105	0.178
Apo-A2, HDL-3	4.95	4.825	0.85	0.452	0.56
Apo-A2, HDL-4	17.26	17.205	0.73	0.157	0.241
		ApoB carrying Lipoprotein Particles (nmol/L)			
Total ApoB Particle Number	1563.38	1514.88	1.07	0.724	0.772
VLDL Particle Number	164.81	171.95	1.67	0.013	0.073
IDL Particle Number	102.14	112.77	1.82	0.004	0.042
LDL Particle Number	1204.73	1176.565	0.86	0.449	0.56
LDL-1 Particle Number	201.68	212.365	1.26	0.286	0.407
LDL-2 Particle Number	157.24	164.32	1.19	0.395	0.515
LDL-3 Particle Number	168.3	172.785	0.95	0.782	0.804
LDL-4 Particle Number	174.6	144.24	0.71	0.105	0.178
LDL-5 Particle Number	212.99	198.84	0.7	0.104	0.178
LDL-6 Particle Number	307.05	312.155	0.89	0.578	0.641

Data availability statement

The data that support the findings of this study are available on reasonable request from the corresponding authors. The data are not publicly available due to privacy and/or ethical restrictions.

Acknowledgments

The authors acknowledge Instruct-ERIC, a Landmark ESFRI project, and specifically the CERM/CIRMMP Italy Infrastructure.

Funding

The Strat- AF study was funded by Tuscany region and Italian Ministry of Health under Grant Aimed Research Call "Bando Ricerca Finalizzata 2013" GR-2013-02355523. The funder had no role in the design, methods, subject recruitment, data collections, analysis or preparation of paper.

A.V. and L.T. acknowledge co-funding from Next Generation EU, in the context of the National Recovery and Resilience Plan, Investment PE8 – Project Age-It: "Ageing Well in an Ageing Society". This resource was co-financed by the Next Generation EU [DM 1557 11.10.2022]. The views and opinions expressed are only those of the authors and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.

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