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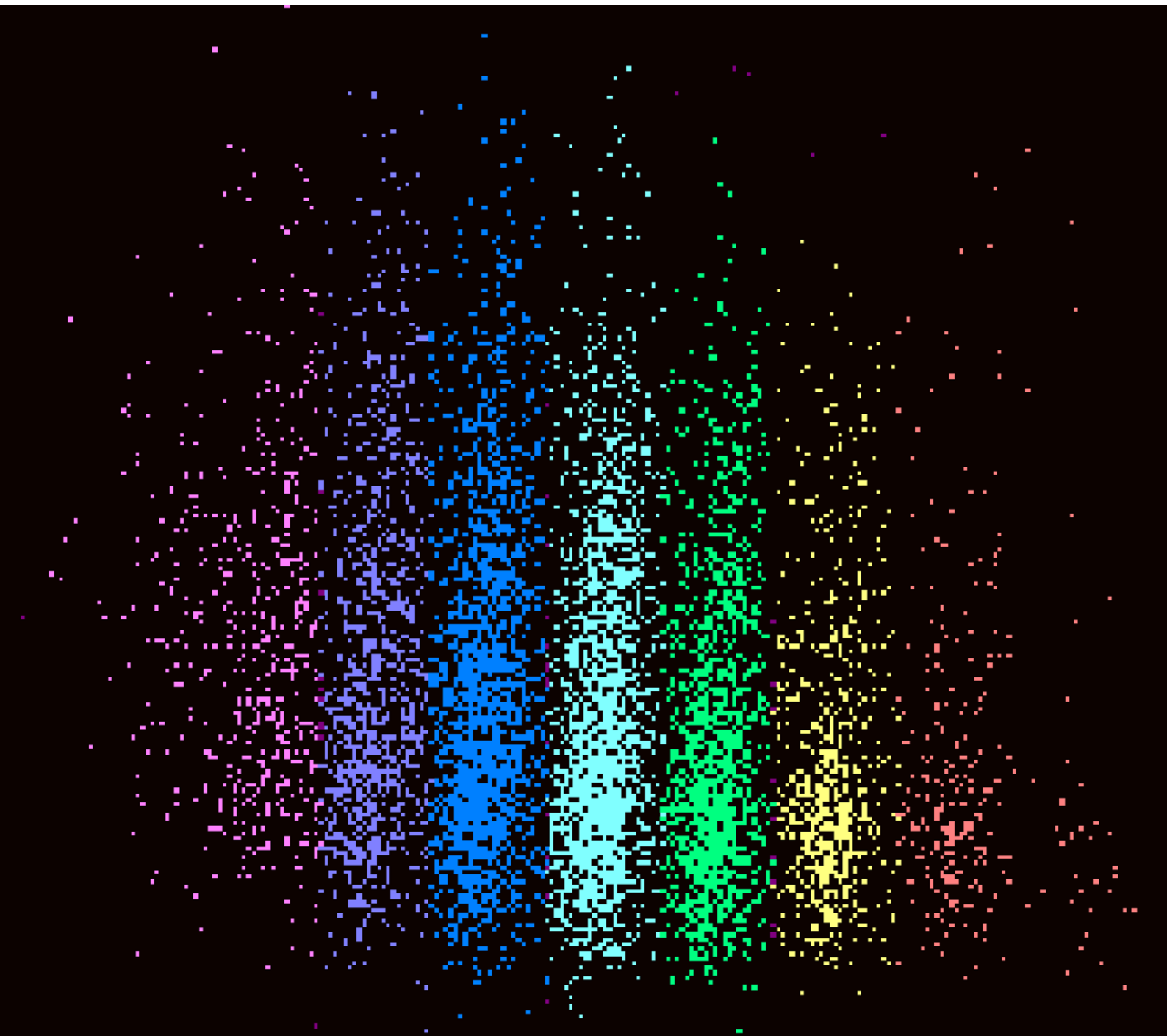

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Contents

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Cytotoxic and dysmetabolic impact of polystyrene nanoplastics, a new potential atherosclerotic cardiovascular risk factor, on a steatosis model of HepG2 cells

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ABSTRACT

The widespread presence of nanoplastics (NPs) in the environment has recently raised concerns regarding the human health. More specifically, adverse effects related to NP exposure and potentially associated with the occurrence and progression of cardiometabolic diseases, including atherosclerotic cardiovascular disease (ASCVD) and metabolic dysfunction-associated steatotic liver disease (MASLD), are currently under investigation. To understand the toxic and dysmetabolic effects induced by NPs in the liver, a major player in cardiometabolic health, we aimed at characterizing the cytotoxic effects induced by polystyrene NPs (PS-NPs) of 500 nm in human hepatocarcinoma HepG2 cells. PS-NPs tested at concentrations of 10, 100, and 200 μg/mL reduced HepG2 cell viability. Intracellular PS-NP content increased according to exposure time and concentration. Moreover, exposure to 500 nm PS-NPs altered glucose uptake after 24 hour-NP exposure (200 μg/mL). This study may contribute to unveil the PS-NP involvement in the pathological mechanisms associated with liver diseases, including MASLD.

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Keywords
Polystyrene nanoplastics; MASLD; ASCVD; cytotoxicity; glucose uptake

Impact of nanoplastics on human health

The intensive industrial development of the last decades brought important changes such as the use of plastic products in our every-day lives [1]. Nowadays, the production of plastics is still increasing [2], as well as the inappropriate recycling and littering of plastic products into the environment [3]. This has led to their degradation under the action of atmospheric agents and the consequent releasing of micro- and nano-plastic fragments throughout the environment and its living organisms [4]. Thereby, humans are subject to microplastics (MPs) and nanoplastics (NPs) exposure both directly and indirectly [5]. Particularly, the constant uncontrolled exposure to NPs has recently raised concerns about the environmental, animal, and human health undermining the One Health approach [6].

A growing body of evidence have shown the presence of MPs and NPs in different human-derived biological matrices, such as blood [7], urine [8] and breast milk [9], as well as in human tissues and organs like testicles [10], ovaries [11], placenta [12], lungs [13] and liver [14].

Moreover, many studies have suggested a role for MPs and NPs in hemolysis and impairment of the coagulation system [15], reproductive function [16] and cardiovascular system [17]. Plastic particles have also been found in human-derived surgically excised carotid artery plaques and correlated with higher risk of myocardial infarction, stroke and death [18]. Indeed, polystyrene-derived NPs (PS-NPs) were also found in the aorta of ApoE $^{-/-}$ mice where they exacerbate the stiffness of the artery and facilitate the formation of the atherosclerotic plaques [19]. Moreover, it was observed that PS-NPs can induce metabolic alterations in macrophages leading them to turn into foam cells, as also usually detected into atherosclerotic plaques [20].

In addition, several studies have investigated the wide spectrum of toxic effects using *in vivo* and *in vitro* animal models [5, 21], and human-derived cell lines [22]. These studies highlighted events like accumulation of plastic particles with different sizes in liver, kidneys and gut, cytotoxicity, DNA damage, increased oxidative stress, inflammation and immune response, neurotoxicity, energy and metabolic disruption. Interestingly, some studies have also reported metabolic dysfunction related to liver toxicity induced by NPs both in aquatic models and mam-

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mals [23]. Since the liver plays a crucial role in the clearance of xenobiotics and its functions support the cardiometabolic health, NPs were proposed as a new potential risk factor involved in liver diseases such as metabolic dysfunction-associated steatotic liver disease (MASLD) [24].

Here we aimed at characterizing the potential cytotoxicity of PS-NPs (500 nm) carrying a negatively charged surface, functionalized with both carboxylic (COO^-) and sulphate (SO_4^-) ions. Our selected NPs were tested in *vitro* in a model of hepatocarcinoma, namely the HepG2 cell line. Cells were treated with PS-NPs for 24 and 48 hours to assess both their viability and NP uptake after exposure. Moreover, we have investigated the ability of HepG2 to uptake glucose (6-NBDG) following NP treatment.

Materials and Methods

Chemicals

MEM with Earle's Salts, Dulbecco's Phosphate Buffered Saline (DPBS), L-Glutamine 100X (200 mM), Minimum Essential Medium Non-Essential Amino Acids Solution (100X), Penicillin/Streptomicin 100X, Sodium Pyruvate 100 mM, and Fetal Bovine Serum (FBS) were purchased from EuroClone S.p.A. (Milan, Italy). Green, red and blue, fluorescent polystyrene nanoparticles (Fluoro-Max Dyed Aqueous Fluorescent Particles) with 500 nm diameter were purchased from Fisher Scientific (20053 Rodano, Italy) as aqueous suspensions at 1% solids by weight and used without any further modification. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and 6-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-Deoxyglucose (6-NBDG) were obtained from Invitrogen (Waltham, MA, USA). Dimethyl sulfoxide (DMSO) was purchased by Thermo Fisher.

Cell culture

The human hepatocarcinoma-derived HepG2 cell line was obtained from the American Type Culture Collection (ATCC®, Manassas, VA, USA) and cultured in MEM previously reconstituted with 10.000 U/mL penicillin, 10 mg/mL streptomycin, non-essential amino acids (0.1 mM), sodium pyruvate (100 mM) and L-glutamine (200 mM) and further supplemented with 10% heat-inactivated FBS. HepG2 were seeded in cell culture petri dishes at a density of $1,5 \times 10^6$ and kept at 37°C with 5% CO_2 for one week. Prior to experiments HepG2 cells were detached with trypsin-EDTA (1X) and seeded into multi-well plates at a different density as follows: 1×10^4 cells/well (MW 96); 1×10^5 cells/well (MW 24).

Succinate dehydrogenase activity (MTT assay)

The MTT assay was performed to assess HepG2 viability after NP treatment. HepG2 were plated in MW96 and incubated at 37°C with 5% CO_2 for 7 days and then treated with polystyrene nanoplastics (PS-NPs) 500 nm at different concentrations (10, 100 and 200 µg/mL) for 24 and 48 hours, respectively. HepG2 were thereby exposed to MTT for 4 hours. MTT-formazan crystals were dissolved in DMSO while keeping the cell culture plates on an orbital shaker (Hosmotic s.r.l., Vico Equense, NA) for 20 minutes. Each plate was read at 570 nm with the EnSpire Multimodel Plate Reader 23001049 (PerkinElmer, MA, USA).

Cellular internalization of 500 nm PS-NPs

To explore the internalization of PS-NPs (500 nm), HepG2 cells were cultured in 24 multi-well plates and exposed to 10, 100 and 200 µg/mL PS-NPs for 24 and 48 hours, respectively. Then, HepG2 cells were washed with DPBS 1X, detached with trypsin-EDTA 1X and re-suspended in DPBS after centrifugation (5 minutes, 2500 rpm). Cytofluorimetric analysis was run with NovoCyte 3000 Flow Cytometer System (Agilent Technologies, CA, USA).

6-NBDG uptake analysis following 500 nm PS-NPs exposure

Prior to 6-NBDG treatment, HepG2 cells were cultured in 24 multi-well plates and exposed to 10, 100 and 200 µg/mL PS-NPs for 24 and 48 hours, respectively. The cell culture medium was thereby replaced by 6-NBDG solution (20 µM) prepared in DPBS 1X, after washing. Cells were incubated for 30 minutes at 37°C with 5% CO_2. After incubation, cells were washed and collected in suitable tubes for cytofluorimetric analysis run by Flow cytometer NovoCyte3000.

Statistical analysis

Statistical analysis was performed using one-way ANOVA and Dunnett multiple test adjustment corrections for normally distributed data. Normality of data distribution was checked by Shapiro-Wilk normality test. P values ≤0.05 were considered significant. Data were shown as mean value ± standard deviation (SD). Statistics was generated using the GraphPad Prism 9.0 software.

Results

500 nm PS-NPs slightly reduced HepG2 cell viability after 24 hours at the highest concentration (200 µg/mL) tested and after 48 hours in all the experimental groups

To evaluate cytotoxic effects induced by 500 nm PS-NPs, MTT assay was performed after exposing HepG2 cells to 500 nm PS-NPs for 24 and 48 hours (**Figure 1**). 500 nm PS-NPs significantly reduced HepG2 cell viability at the highest concentration (200 µg/mL) following 24 hours of treatment. HepG2 cell viability was significantly reduced at all tested concentrations after 48 hours. Moreover, at the highest concentration (200 µg/mL), after 48 hour-treatment, HepG2 cell viability resulted higher compared to the other tested concentrations although statistically lower than the control group. This latter could be potentially related to the exit of PS-NPs from HepG2 cells as time of exposure to 500 nm PS-Ns increases. The reduction of HepG2 cell viability was maintained around the 80% threshold (dashed line, highlighted in red), considered the actual threshold below which cell viability is substantially impaired.

500 nm PS-NPs are taken up in a dose-response fashion by HepG2 cells after 24 and 48 hours

To evaluate the cellular internalization of 500 nm PS-NPs, we have performed a cytofluorimetric analysis by exploiting the fluorophore coated to PS-NPs (**Figure 2**). HepG2 cells were exposed to fluorescent 500 nm PS-NPs for 24 and 48 hours and the fluorescent signal was detected by cytofluorometric analysis. 500 nm PS-NPs were taken up in a dose-response manner both after 24 and 48 hours of treatment. More specifically, it was observed an increase in the uptake of 500 nm PS-NPs after 24 hour-exposure in HepG2 cells of +1190%, +8543% and +9991% for each concentration (10, 100 and 200 µg/mL, respectively) compared to the control group. Conversely, after 48 hours of exposure, 500 nm PS-NPs uptake in HepG2 cells increased of +1356%, +5882% and +8014% for each concentration (10, 100 and 200 µg/mL, respectively) compared to the control group. The uptake appeared lower after 48 hour-treatment at the middle and highest concentrations (i.e., 100 and 200 µg/mL) with respect to the 24 hour-treatment. Possibly, this could be explained by the potential exit of 500 nm PS-NPs from HepG2 cells.

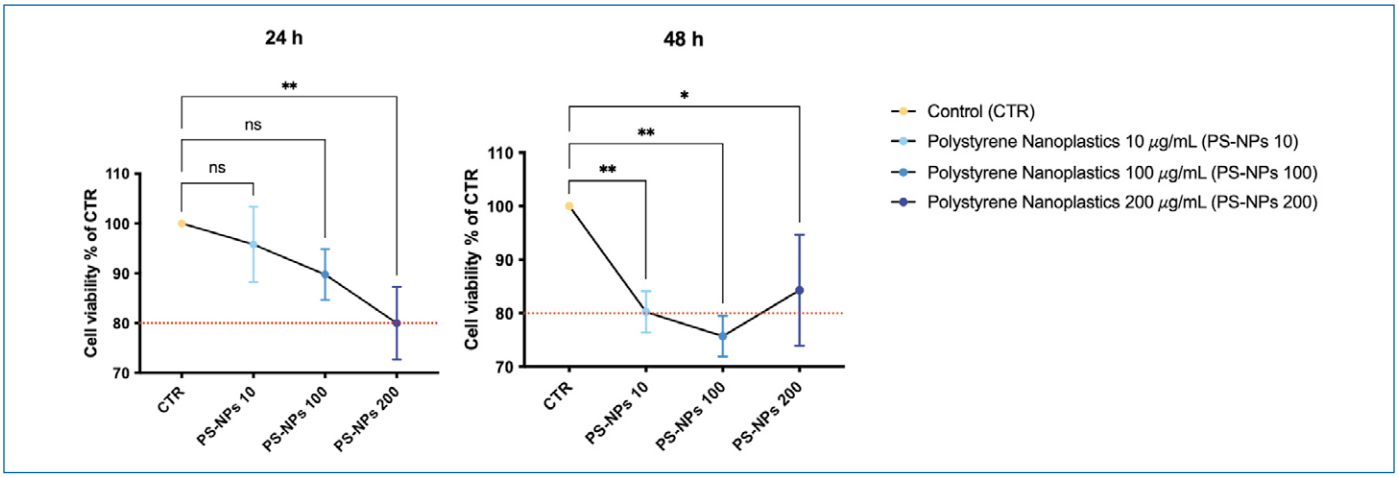

Figure 1 | Effects of PS-NPs on cell viability (MTT Assay). Each point of the linear regression curve reports mean value ± SD obtained from 3 independent experiments, each run in quintuplicates. CTR=100%. Viability threshold: 80%. *p<0.05, **p<0.01 (One-way ANOVA followed by Dunnett's multiple comparison correction).

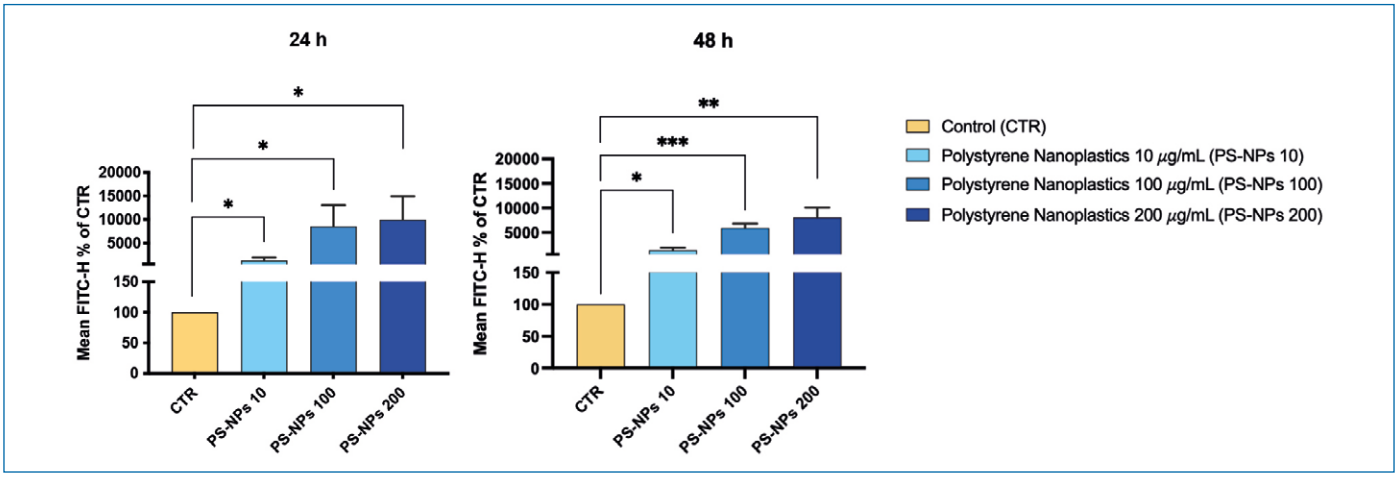

Figure 2 | PS-NPs internalization by HepG2 cells (cytofluorimetry). MFI = mean fluorescence intensity. Each bar reports mean value ± SD obtained from 3 independent experiments each run in triplicates. CTR=100%. *p<0.05, ** p<0.01, ***p<0.001 (Unpaired t-test).

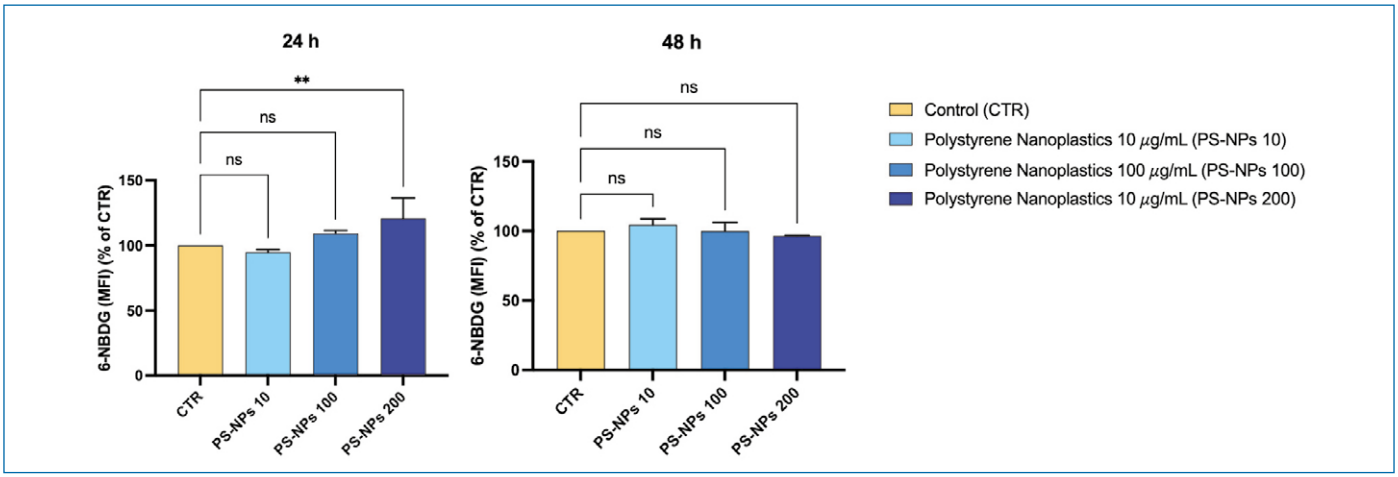

Figure 3 | Effects of PS-NPs on 6-NBDG uptake by HepG2 cells (cytofluorimetry). MFI = mean fluorescence intensity. 6-NBDG: 20 μM (30 minutes). Each bar reports mean value ± SD obtained from 3 independent experiments each run in triplicate. "ns": non-significant; **p<0.01 (24 h: One-way ANOVA followed by Dunnett's multiple comparison correction; 48 h: Kruskal-Wallis followed by Dunnett's multiple comparison correction).

6-NBDG uptake in HepG2 cells was not affected after 24 and 48 hours of 500 nm PS-NPs exposure

The effect of exposure to 500 nm PS-NPs on 6-NBDG uptake by HepG2 cells was evaluated (**Figure 3**). No significant effects on 6-NBDG uptake were observed after 24/48 hours of treatment, except for the highest concentration (200 µg/mL) after 24 hour-treatment. However, after 24 hour-treatment with 500 nm PS-NPs, it was also observed a trend in increasing uptake of 6-NBDG uptake by HepG2 cells in a dose-response fashion. The increase of glucose uptake in HepG2 could be due to the stress response induced by exposure to 500 nm PS-NPs in the acute phase of treatment. Conversely, the same trend in increasing glucose uptake was not observed after 48 hour-treatment with 500 nm PS-NPs. In this latter, glucose uptake in HepG2 cells appeared to decrease in a dose-response manner compared to 24 hours of exposure to 500 nm PS-NPs. Possibly, this could suggest that glucose uptake in HepG2 cells may decrease in a time-dependent manner, as the exposure time to 500 nm PS-NPs is associated with increased metabolic impairment.

Discussion

In light of the potential cytotoxic effects of PS-NPs, specifically to the liver, we started to address this issue by exposing human HepG2 cells to 500 nm PS-NPs and found effects on cell viability and accumulation of PS-NPs, while glucose uptake appeared only slightly affected. The observed reduction of cell viability did not exceed 20%. Nevertheless, it is known that NPs affect cell viability differently according to cell types, plastic polymers, sizes or functional surface groups characterizing the plastic particles [25-27]. Compared to MPs, NPs are characterized by a greater surface-to-volume ratio and surface properties allowing them to carry environmental pollutants and microorganisms, thus exerting a "Trojan horse" effect [28]. Herein we observed a reduced intracellular content of 500 nm PS-NPs in HepG2 especially after 48 hour-exposure, possibly due to passive release of PS-NPs. Accordingly, Liu et al. have shown that 500 nm PS-NPs are taken up by rat basophilic leukemia cells mainly through micropinocytosis and excreted using both passive penetration and active lysosomal exocytosis [29]. In a different study, based on mouse embryonic fibroblasts, PS-NPs clearance occurred mainly via exocytosis when the retrograde intracellular transport was inhibited [30]. To our knowledge, the mechanisms modulating PS-NPs dynamics in HepG2 cells were not yet investigated. PS-NPs were also found to induce metabolic dysfunctions linked to both glucose and lipid metabolisms, as observed in animal models [31]. Here, we speculated that potential metabolic alterations associated with impairment of the cellular glucose uptake were not observed considering the acute exposure time to 500 nm PS-NPs. Hence, time of treatment or dosages should be increased to detect relevant metabolic impairments in HepG2 cells, as it was seen in animal models. Recent studies based on animal models have also described how PS-NPs can contribute to adipose tissue dysfunctions [32], atherosclerosis [19], vascular disease [33] and MASLD [23]. However, the pathological mechanisms linking PS-NP-induced hepatic dysfunctions to ASCVD remain to be elucidated. To this purpose, future studies will investigate PS-NP effects in a steatotic-like model of HepG2 cells recapitulating key features of MASLD.

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Author Contributions

Conceptualization, C.G., L.D.D., P.M.; methodology, C.G., L.C., F.T.K., L.D. and, P.M.; validation, C.G L.C. and P.M.; investigation, C.G.; data curation, C.G; writing-original draft preparation, C.G; writing-review and editing, C.G., L.C., and P.M.; supervision, P.M.; project administration, P.M.; funding acquisition, P.M. All authors have read and agreed to the published version of the manuscript.

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Competing interests

All the authors have nothing to disclose.

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Effects of fibrates on lipid profile: a meta-analysis of randomized controlled trials

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ABSTRACT

We conducted a meta-analysis to compare the lipid-lowering effects of fibratesLiterature was searched up to December 2024. Absolute changes in triglycerides (TG), LDL-cholesterol (LDL-C), apolipoprotein B (ApoB), and non-HDL cholesterol (non-HDL-C) were analyzed using both fixed and random effects models.

We included 63 randomized controlled trials. Compared to placebo, all fibrates significantly reduced TG levels. Fenofibrate and bezafibrate significantly reduced LDL-C (–15.12 and –15.04 mg/dL, respectively), while only fenofibrate significantly lowered ApoB (–24.88 mg/dL) and non-HDL-C (–46.38 mg/dL), followed by gemfibrozil and pemafibrate for non-HDL-C. In combination with statins, no fibrates significantly reduced LDL-C, while fenofibrate remained the only fibrate to significantly lower ApoB (–10.42 mg/dL) and non-HDL-C (–12.02 mg/dL).

Overall, we found that fibrates differ substantially in their lipid effects. Fenofibrate shows the most consistent and comprehensive lipid-lowering profile.

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Keywords
Fibrates; Mixed dyslipidemia; Dyslipidemia; Fibrates; Lipid-lowering therapy

Background

Dyslipidemia is a major modifiable risk factor for cardiovascular disease and remains a leading target for both primary and secondary prevention strategies. While elevated low-density lipoprotein cholesterol (LDL-C) is the primary focus of lipid-lowering therapies, patients with dyslipidemia often present with a complex lipid profile that involves abnormalities in multiple lipid parameters. These may include elevated triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), and increased concentrations of apolipoprotein B (ApoB) [1, 2], each of which is independently associated with increased cardiovascular risk.

In clinical practice, this multifaceted dyslipidemic profile is particularly common in patients with metabolic syndrome, type 2 diabetes mellitus, and obesity [3-5]. In these populations, isolated LDL-C lowering may be insufficient to achieve optimal cardiovascular protection. Consequently, there is growing recognition of the need for comprehensive lipid management that targets a broader range of lipid abnormalities. This has led to increasing use of combination therapies that go beyond statin monotherapy [5, 6].

Statins, or HMG-CoA reductase inhibitors, are the cornerstone of lipid-lowering treatment and are highly effective in reducing LDL-C and cardiovascular events. However, their effect on triglyceride levels is moderate and variable, and they have minimal or no impact on HDL-C or ApoB in many patients. Fibrates, are a class of lipid-modifying agents that act primarily as peroxisome proliferator-activated receptor alpha (PPARα) agonists. They are particularly effective in lowering triglyceride levels and, to a lesser extent, in raising HDL-C [7]. Some fibrates also modestly reduce LDL-C and ApoB levels [7, 8]. Because of their complementary lipid effects, fibrates are often considered as add-on therapy to statins in patients with mixed dyslipidemia, especially when elevated triglycerides and low HDL-C persist despite statin use [9].

Despite their shared pharmacologic class, fibrates differ significantly in their pharmacokinetics, receptor selectivity, potency, and lipid-lowering profiles [10, 11]. Moreover, their interaction profiles with statins are not uniform, which has implications for both efficacy and safety, particularly in the context of combination therapy [12].

Despite compelling evidence for the utility of fibrates, comparisons among individual agents in randomized clinical trials remain scarce. Absent such direct comparisons, it is unclear whether observed differences are due to pharmacodynamic distinctions or co-

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hort selection biases. Given these differences, therapeutic decisions involving fibrates – whether as monotherapy or in combination with statins – should not treat the class as homogenous. Instead, clinicians should consider the specific pharmacological and clinical characteristics of each molecule. A nuanced understanding of these differences is crucial for optimizing lipid management strategies in patients with complex dyslipidemia, especially those with residual lipid abnormalities despite statin therapy [13].

In this context, the present meta-analysis examined 63 randomized controlled trials comparing fibrates versus placebo, or fibrate-plus-statin versus statin alone. Our goal was to quantify and contrast the magnitude of lipid changes – specifically TG, LDL-C, ApoB, and non-HDL-cholesterol (non-HDL-C – across different fibrates, both as monotherapy and combination therapy.

Methods

We conducted a meta-analysis according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [14].

Search Strategy and Information Sources

PubMed, EMBASE, Web of Science were searched from inception to December 2024. Details of searching strategies are shown in the **Supplementary material**.

Study Selection and Eligibility Criteria

Inclusion criteria were:

1) randomized controlled trials (RCTs) in humans, parallel design, phase II, III, or IV;
2) English language and full text available;
3) comparing the effect of fibrates both alone versus placebo and on top of statins versus statins;
4) reporting the absolute effects on TG, LDL-C, ApoB, or non-HDL-C levels.

Data extraction and synthesis

Two independent investigators extracted the data using a predefined data collection form including the first author; year of publication; the number of participants and their main characteristics (e.g. sex, mean age); intervention duration; treatment (name and dosage) and control; mean or median values and variance (standard deviation [SD], standard error [SE], interquartile range [IQR], 95% confidence interval [95%CI], the minimum and maximum values [range]) both at baseline and follow-up or absolute change for TG, LDL-C, ApoB, and non-HDL-C concentrations. The between-group (treatment vs. placebo) absolute mean differences in TG, LDL-C, ApoB, and non-HDL-C levels, along with their 95% confidence intervals, were calculated for each fibrate. All lipid values were expressed in mg/dL; when reported in mmol/L, they were converted using standard factors: values for TG were divided by 0.0113, while LDL-C, ApoB, and non-HDL-C were converted using a factor of 0.0259.

All data were presented as mean and SD. We converted SE, IQR, 95%CI, and range to SD by using formulas recommended by the Cochrane Handbook [15]. Since the within-group absolute mean difference was computed by subtracting the baseline level from the follow-up level, 0.5 was used as the correlation coefficient to calculate pooled SD within groups [16]. For trials that reported variances at baseline but without any information for variances at follow-up, the variances at baseline were also used for follow-up. Multiple intervention groups were combined into a single intervention group when they were compared to only one control group in the trial. Pooled estimates were assessed by using both the fixed-effects and the random-effects models. The generic inverse variance method was used to balance the heterogeneity between studies, and the restricted maximum likelihood estimator was used to estimate the between-study variance [17]. When significant heterogeneity was discovered (as determined by Cochrane's Q test and the I^2 statistic [18], $p<0.05$), the results from the random-effects model were presented.

All tests were considered statistically significant for p-value less than 0.05. The analyses and the corresponding graphical visualization of forest plots were conducted using R (version 4.3.2.).

Results

The flow chart indicating the procedure of literature searching and study screening is shown in **Supplementary Figure 1**. A total of 33,333 subjects from 63 RCTs were included in our meta-analysis (9 RCTs for bezafibrate, 3 RCTs for ciprofibrate, 3 RCTs for clofibrate, 19 RCTs for fenofibrate, 23 RCTs for gemfibrozil, 6 RCTs for pemafibrate, **Table 1**).

Figure 1 summarizes the lipid effects of different fibrates compared to placebo, without concomitant statin treatment. With the exception of clofibrate, fibrates significantly reduced TG levels (**Figure 1A**), with the greatest reduction observed for pemafibrate (–123.91 mg/dL [-196.60; -51.22]), followed by gemfibrozil (–93.22 mg/dL [-112.76; -73.69]), ciprofibrate (–75.05 mg/dL [-113.96; -36.14]), bezafibrate (–65.87 mg/dL [-95.25; -36.48]), and fenofibrate (–64.81 mg/dL [-83.90; -45.71]) (**Figure 1A**).

For LDL-C, significant reductions were observed with fenofibrate (–15.12 mg/dL [-29.89; -0.34]) and bezafibrate (–15.04 mg/dL [-21.92; -8.16]). All other fibrates showed no significant effect (**Figure 1B**).

Regarding ApoB, fenofibrate showed the most marked reduction (–24.88 mg/dL [-38.73; -11.03]), followed by bezafibrate (–20.81 mg/dL [-33.85; -7.78]), and gemfibrozil (–12.01 mg/dL [-18.16; -5.86]), while other fibrates had non-significant effects (**Figure 1C**).

In the case of non-HDL-C, only three fibrates had enough RCTs to assess a pooled effect, and all demonstrated significant reductions versus placebo: fenofibrate (–46.38 mg/dL [-61.50; -31.26]), gemfibrozil (–33.89 mg/dL [-36.02; -31.75]), and pemafibrate (–17.40 mg/dL [-23.86; -10.95]) (**Figure 1D**).

Figure 2 shows the effects of fibrates on top of statin therapy. This evaluation was possible only for fenofibrate and gemfibrozil, as they were the only fibrates with more than one eligible trial. In both cases, a significant reduction in TG levels was observed, with decreases of –59.09 mg/dL [-78.99; -39.20] and –44.68 mg/dL [-63.64; -25.72], respectively (**Figure 2A**). When considered on top of statin therapy, no fibrate class significantly reduced LDL-C levels. On the contrary, pemafibrate was associated with a significant increase (+9.68 mg/dL [8.48; 10.89]) (**Figure 2B**). Fenofibrate remained the only fibrate associated with a significant reduction in ApoB levels (–10.42 mg/dL [-15.17; -5.67]) (**Figure 2C**) and with a modest reduction in non-HDL-C (–12.02 mg/dL [-15.17; -5.67]), with no consistent effect observed for the other fibrates (**Figure 2D**).

Discussions

This meta-analysis of 63 randomized controlled trials including over 33,000 participants provides compelling evidence that fibrates exert heterogeneous effects on lipid parameters, with significant differences among individual agents.

While all fibrates significantly reduced TG levels, fenofibrate and

Table 1 | Characteristics of included trials.

No	Trial name	Year	Experimental group	Control group	Number of participants
Bezafibrate					
1	Niort et al (1988)[s1]	1988	Bezafibrate 400 mg/day	Placebo	24
2	Jones et al (1990)[s2]	1990	Bezafibrate 600 mg/day	Placebo	37
3	Winocour et al (1990)[s3]	1990	Bezafibrate 400 mg/day	Placebo	36
4	Niort et al (1993)[s4]	1993	Bezafibrate 400 mg/day	Placebo	32
5	Walzl et al (1993)[s5]	1993	Bezafibrate 400 mg/day	Placebo	40
6	Stewart et al (1995)[s6]	1995	Bezafibrate 400 mg/day	Placebo	22
7	SENDCAP[s7]	1998	Bezafibrate 400 mg/day	Placebo	128
8	Ogawa et al (2000)[s8]	2000	Bezafibrate 400 mg/day	Placebo	342
9	Leon-Martinez et al (2020)[s9]	2020	Bezafibrate 400 mg/day + Berberine 500 mg/day	Berberine 500 mg/day	20
Ciprofibrate					
1	Illingworth et al (1982)[s13]	1982	Ciprofibrate 50 or 100 mg/day	Placebo	20
2	Kontopoulos et al (1996)[s14]	1996	Ciprofibrate 100 mg/day + Simvastatin 20 mg/day	Simvastatin 20 mg/day	40
3	Bermudez-Pirela et al (2007)[15]	2007	Ciprofibrate 100 mg/day	Placebo	75
Clofibrate					
1	Cole et al (1971)[s10]	1971	Clofibrate 0.25 g/day	Placebo	119
2	Dujovne et al (1976)[s11]	1976	Clofibrate 2 g/day	Placebo	19
3	Miettinen et al (1980)[s12]	1980	Clofibrate 1.5 g/day or + Probucol 1-2 g/day	Placebo or + Probucol 1-2 g/day	100
Fenofibrate					
1	Mellies et al (1987)[s16]	1987	Fenofibrate 300 mg/day	Placebo	33
2	Athyros et al (2002)[s17]	2002	Fenofibrate 200 mg/day + Atorvastatin 20 mg/day	Atorvastatin 20 mg/day	80
3	Playford et al (2002)[s18]	2002	Fenofibrate 200 mg/day	Placebo	35
4	Cavallero et al (2003)[s19]	2003	Fenofibrate 200 mg/day	Placebo	28
5	Playford et al (2003)[s20]	2003	Fenofibrate 200 mg/day	Placebo	35
6	Vakkilainen et al (2003)[s21]	2003	Fenofibrate 200 mg/day	Placebo	405
7	Derosa et al (2004)[s22]	2004	Fenofibrate 200 mg/day + Fluvastatin 80 mg/day	Fluvastatin 80 mg/day	48
8	Athyros et al (2005)[s23]	2005	Fenofibrate 200 mg/day + Atorvastatin 20 mg/day	Atorvastatin 20 mg/day	200
9	Okopien et al (2005)[s24]	2005	Fenofibrate 267 mg/day	Placebo	34
10	Keech et al (2005)[s25]	2005	Fenofibrate 200 mg/day	Placebo	9795
11	Davidson et al (2009)[s26]	2009	Fenofibrate 145 mg/day + Atorvastatin 40 mg/day	Atorvastatin 40 mg/day	137
12	Derosa et al (2009)[s27]	2009	Fenofibrate 145 mg/day + Simvastatin 40 mg/day	Simvastatin 40 mg/day	153
13	Farnier et al (2010)[s28]	2010	Fenofibrate 160 mg/day + Pravastatin 40 mg/day	Pravastatin 40 mg/day	239
14	Miyazaki et al (2010)[s29]	2010	Fenofibrate 300 mg/day	Placebo	44
15	Krysiak et al (2011)[s30]	2011	Fenofibrate 200 mg/day alone or + Simvastatin 40 mg/day	Placebo or + Simvastatin 40 mg/day	190
16	Lella et al (2013)[s31]	2013	Fenofibrate 145 mg/day + Atorvastatin 10 mg/day	Atorvastatin 10 mg/day	58

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No	Trial name	Year	Experimental group	Control group	Number of participants
17	La Fountaine et al (2019)[s32]	2019	Fenofibrate 145 mg/day	Placebo	16
18	Ihm et al (2020)[s33]	2020	Fenofibrate 160 mg/day + Pitavastatin 2 mg/day	Pitavastatin 2 mg/day	347
19	Park et al (2021)[s34]	2021	Fenofibrate 178.8 mg/day + Atorvastatin 10 or 20 or Rosuvastatin 10 mg/day	Atorvastatin 10 or 20 or Rosuvastatin 10 mg/day	127
Gemfibrozil					
1	HHS[s35]	1987	Gemfibrozil 1200 mg/day	Placebo	4081
2	Andersen et al (1990)[s36]	1990	Gemfibrozil 1200 mg/day	Placebo	43
3	Tsai et al (1992)[s37]	1992	Gemfibrozil 1200 mg/day	Placebo	12
4	Lahdenpera et al (1993)[s38]	1993	Gemfibrozil 1200 mg/day	Placebo	16
5	Wiklund et al (1993)[s39]	1993	Gemfibrozil 1200 mg/day alone or + Pravastatin 40 mg	Placebo or + Pravastatin 40 mg	266
6	Vinik et al (1993)[s40]	1993	Gemfibrozil 1200 mg/day	Placebo	442
7	Vuorinen-Markkola et al (1993)[s41]	1993	Gemfibrozil 1200 mg/day	Placebo	20
8	Knipscheer et al (1994)[s42]	1994	Gemfibrozil 1200 mg/day	Placebo	33
9	Avellone et al (1995)[s43]	1995	Gemfibrozil 1200 mg/day	Placebo	20
10	Kahri et al (1995)[s44]	1995	Gemfibrozil 1200 mg/day	Placebo	20
11	Smit et al (1995)[s45]	1995	Gemfibrozil 1200 mg/day + Fluvastatin 40 mg/day	Fluvastatin 40 mg/day	14
12	Vanhanen et al (1995)[s46]	1995	Gemfibrozil 1200 mg/day alone or + Pravastatin 40 mg/day	Placebo or + Pravastatin 40 mg/day	38
13	Sane et al (1995)[s47]	1995	Gemfibrozil 1200 mg/day	Placebo	20
14	Schaefe et al (1996)[s48]	1996	Gemfibrozil 1200 mg/day	Placebo	229
15	LOCAT[s49]	1997	Gemfibrozil 1200 mg/day	Placebo	395
16	Yoshida et al (1998)[s50]	1998	Gemfibrozil 900 mg/day	Placebo	19
17	VA-HIT[s51]	1999	Gemfibrozil 1200 mg/day	Placebo	2531
18	Avogaro et al (1999)[s52]	1999	Gemfibrozil 1200 mg/day	Placebo	217
19	Mussoni et al (2000)[s53]	2000	Gemfibrozil 1200 mg/day	Placebo	53
20	Dumont et al (2001)[s54]	2001	Gemfibrozil 1200 mg/day	Placebo	64
21	Bosse et al (2002)[s55]	2002	Gemfibrozil 1200 mg/day	Placebo	65
22	Miller et al (2002)[s56]	2002	Gemfibrozil 1200 mg/day	Placebo	37
23	Martínez et al (2003)[s57]	2003	Gemfibrozil 1200 mg/day	Placebo	70
Pemafibrate					
1	Ishibashi et al (2016)[s58]	2016	Pemafibrate 0.05 or 0.1 or 0.2 or 0.4 mg/day	Placebo	178
2	Arai et al (2017)[s59]	2017	Pemafibrate 0.1 or 0.2 or 0.4 mg/day + Pitavastatin or Pemafibrate 0.2 or 0.4 mg/day + Any statins	Pitavastatin or Any statins	593
3	Arai et al (2018)[s60]	2018	Pemafibrate 0.1 or 0.2 or 0.4 mg/day	Placebo	166
4	Matsuba et al (2018)[s61]	2018	Pemafibrate 0.4 mg/day	Placebo	18
5	Nakajima et al (2021)[s62]	2021	Pemafibrate 0.4 mg/day	Placebo	118
6	PROMINENT[s63]	2022	Pemafibrate 0.4 mg/day + Any statins	Placebo + Any statins	10497

*The references for each trial can be found in the supplementary file.

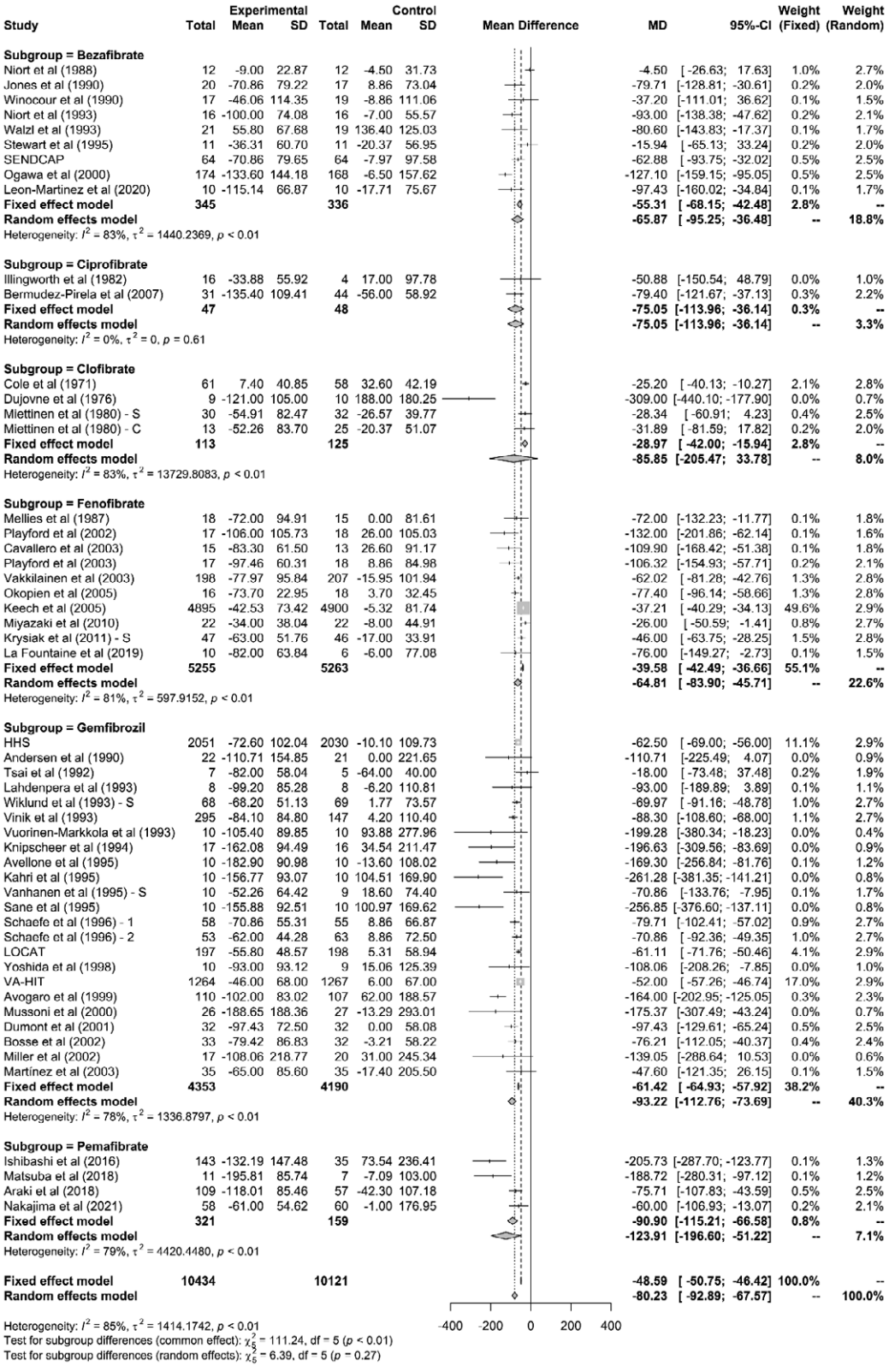

Figure 1A | Lipid-lowering effects of fibrates compared to placebo. Panel A: effects on triglycerides (TG); Panel B: effects on low-density lipoprotein cholesterol (LDL-C); Panel C: effects on apolipoprotein B (ApoB); Panel D: effects on non-high-density lipoprotein cholesterol (non-HDL-C). Effects on triglycerides (TG).

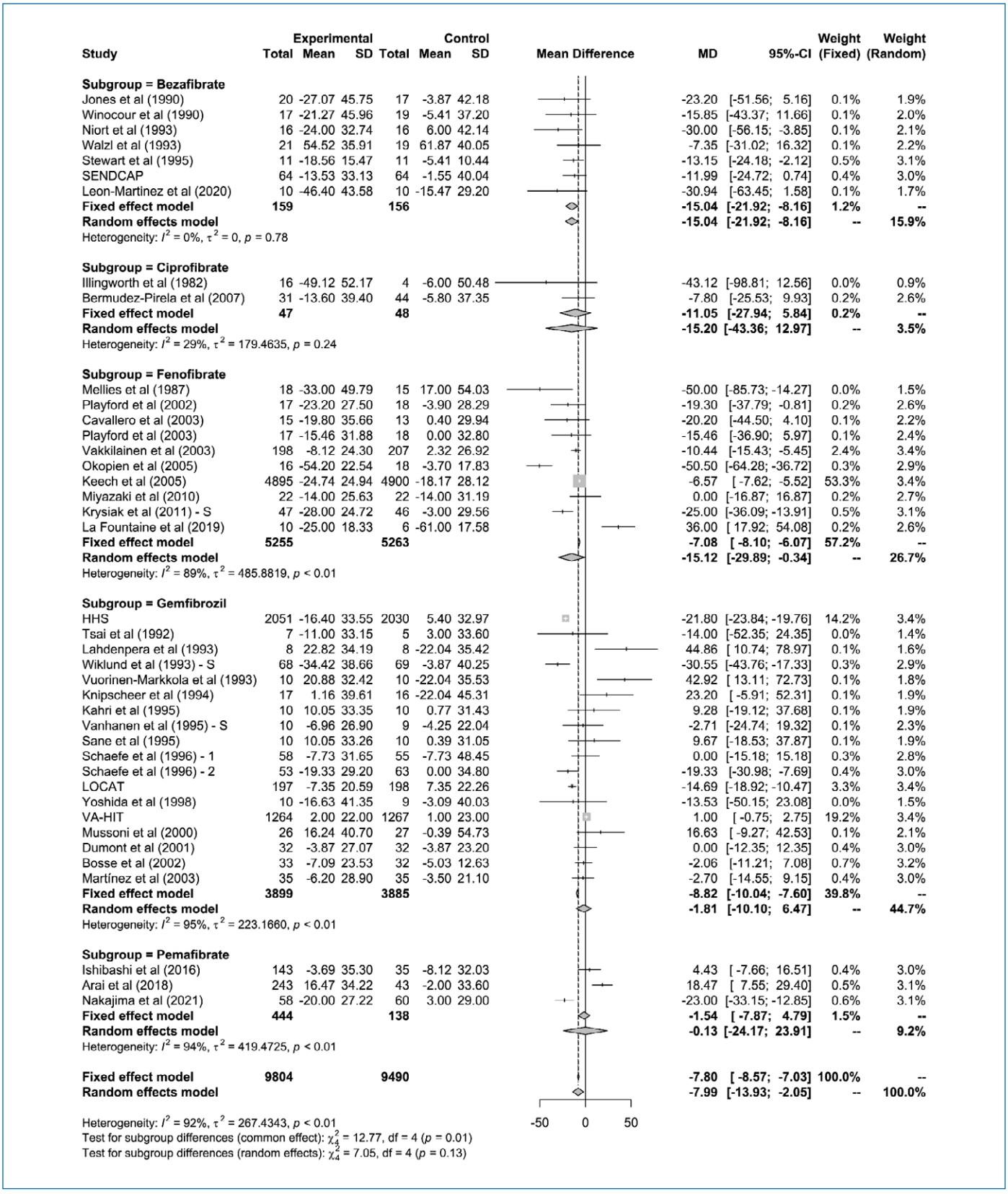

Figure 1B | Effects on low-density lipoprotein cholesterol (LDL-C).

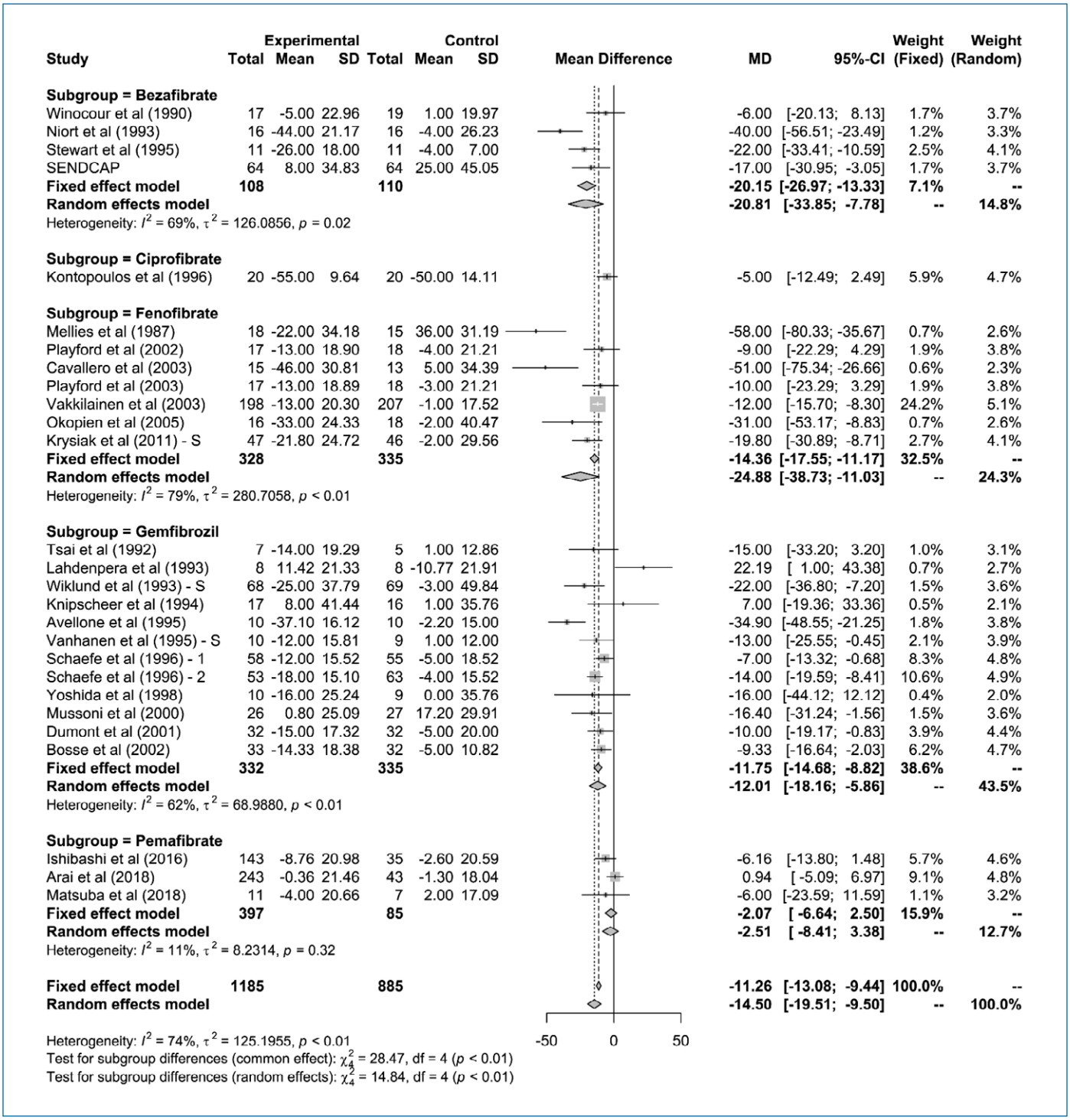

Figure 1C | Effects on apolipoprotein B (ApoB).

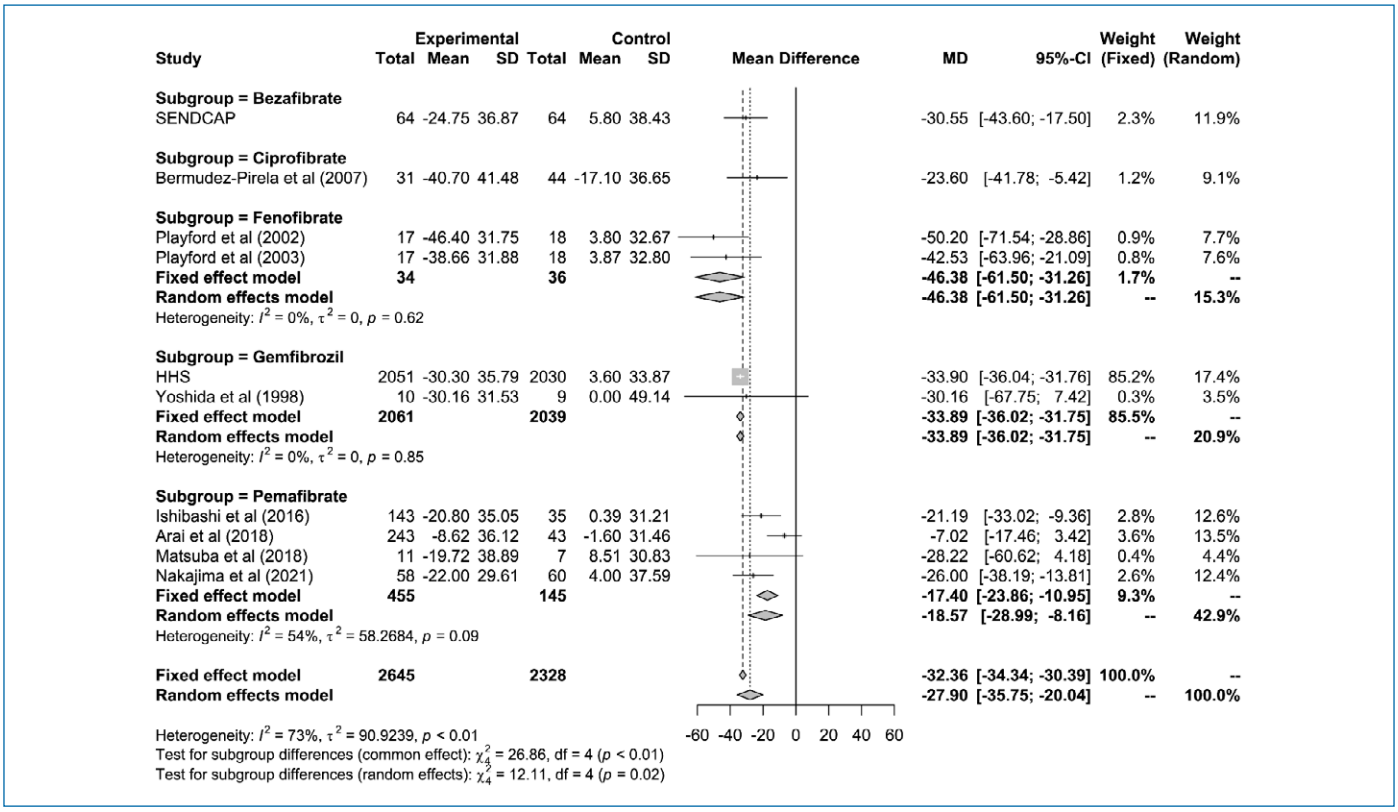

Figure 1D | Effects on non-high-density lipoprotein cholesterol (non-HDL-C).

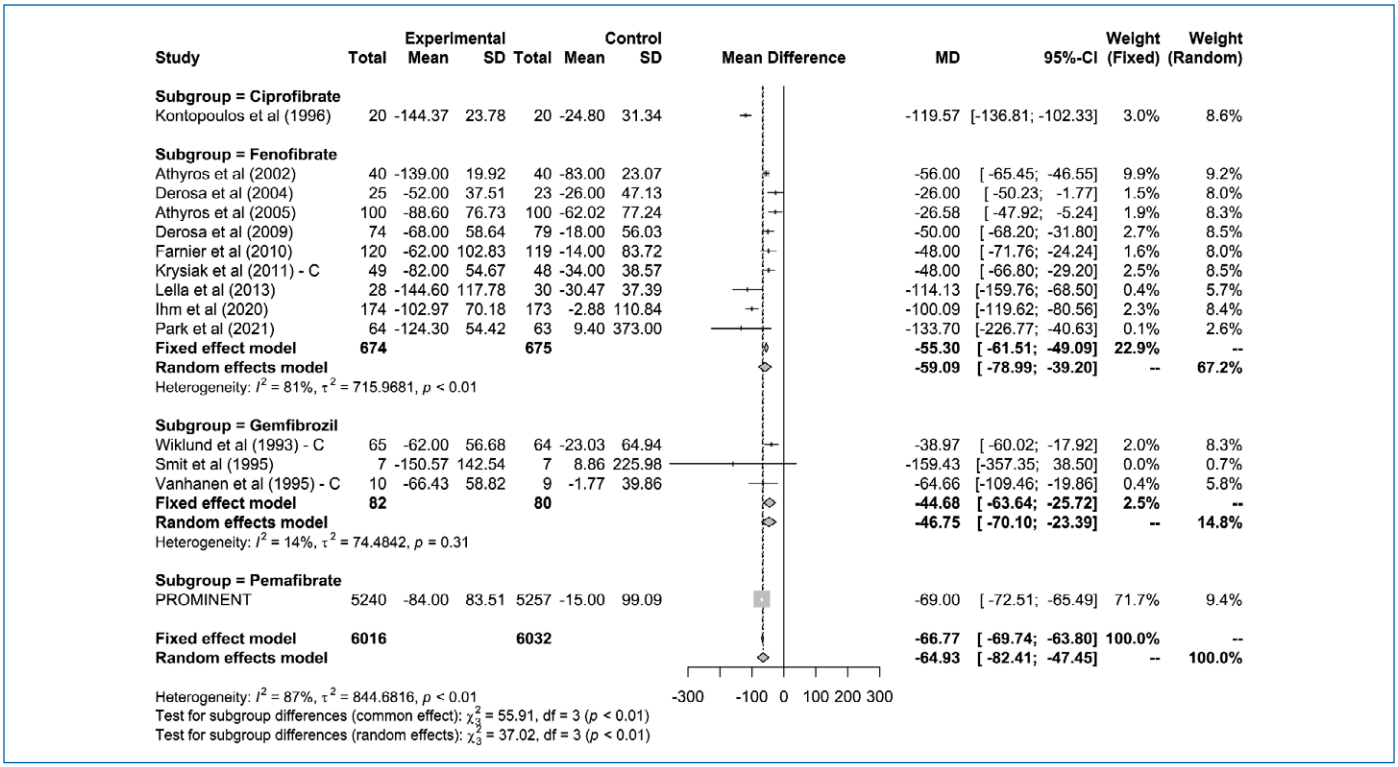

Figure 2A | Lipid-modifying effects of fibrates added to statin therapy. Panel A: effects on triglycerides (TG); panel B: effects on low-density lipoprotein cholesterol (LDL-C); panel C: effects on apolipoprotein B (ApoB); Panel D: effects on non-high-density lipoprotein cholesterol (non-HDL-C). Effects on triglycerides (TG).

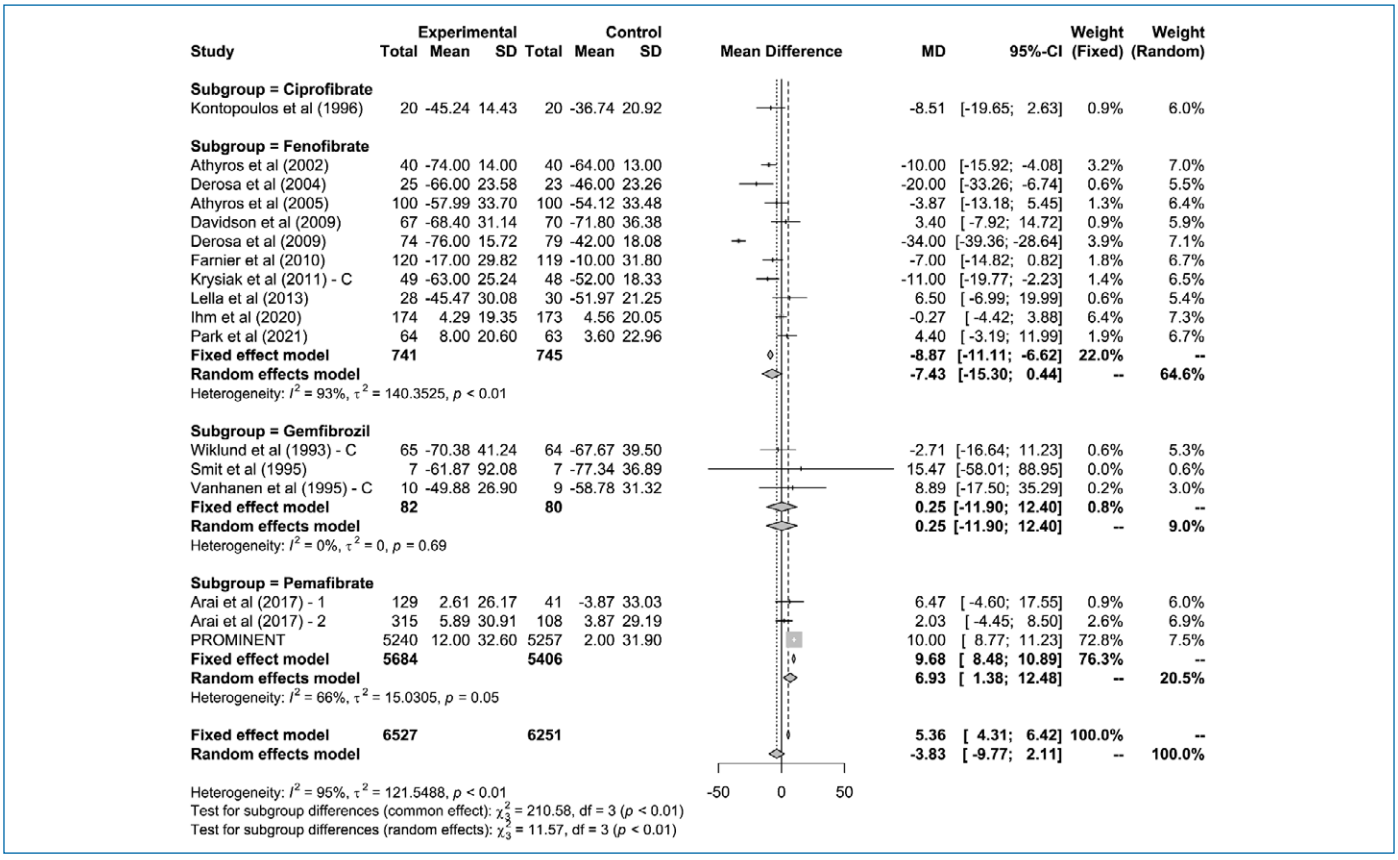

Figure 2B | Effects on low-density lipoprotein cholesterol (LDL-C).

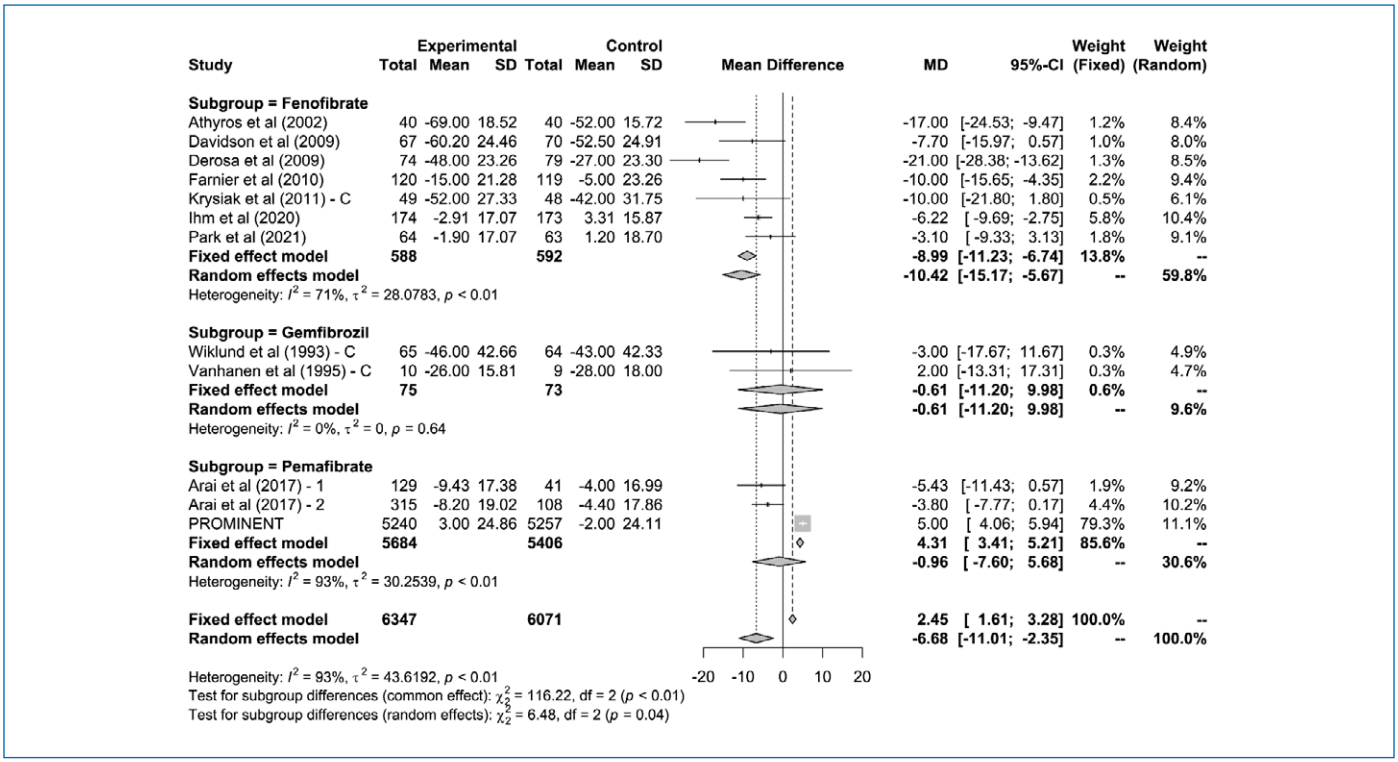

Figure 2C | Effects on apolipoprotein B (ApoB).

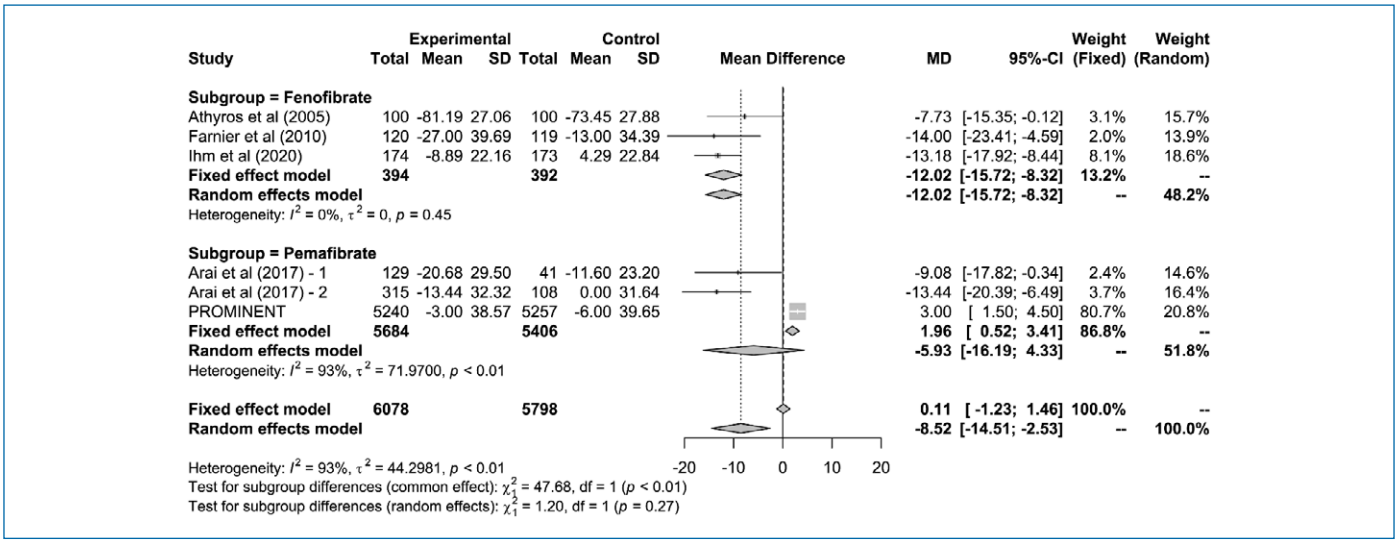

Figure 2D | Effects on non-high-density lipoprotein cholesterol (non-HDL-C).

bezafibrate consistently lowered both LDL-C and ApoB, but only fenofibrate confirmed these effects whether used as monotherapy or in combination with statins.

These findings support the hypothesis that not all fibrates are pharmacologically or clinically equivalent. Despite sharing the PPAR-α agonist mechanism, fibrates differ in their affinity for nuclear receptors, tissue-specific activity, and effects on lipid-modifying gene expression. Fenofibrate's capacity to reduce both ApoB and LDL-C suggests broader atherogenic lipoprotein modulation, which may underlie the subgroup benefits observed in large cardiovascular outcome trials such as FIELD and ACCORD-Lipid, particularly among patients with atherogenic dyslipidemia [19, 20].

Regarding pemafibrate, despite its favorable impact on TG levels, it failed to lower – and even increased – LDL-C levels in the statin-combination subgroup. These findings may in part explain the neutral results of the PROMINENT trial [21], where pemafibrate did not reduce major cardiovascular events in patients with type 2 diabetes and atherogenic dyslipidemia despite significant TG lowering. This reinforces the notion that reduction in TGs alone, without corresponding improvement in ApoB or LDL-C, may be insufficient to achieve cardiovascular risk reduction.

Interestingly, gemfibrozil showed moderate ApoB reduction but had no significant effect on LDL-C. Historical trials (HHS [22], VA-HIT [23]) showed cardiovascular benefit with gemfibrozil, but their results predate modern statin use and may reflect unique metabolic effects, including modulation of HDL particle functionality [24], which were not captured in our lipid-focused analysis. Gemfibrozil was shown to effectively reduce TG, ApoB, and non-HDL cholesterol, but its use in combination with statins is contraindicated, as leads to increased plasma levels of statins, raising the risk of severe myopathy and rhabdomyolysis [12].

Limitations

Several limitations of our analysis warrant mention. First, we included trials with varying durations, populations, and background therapies, which may contribute to heterogeneity. Second, few trials directly comparing fibrates were available; thus, comparisons among agents rely on indirect data. Third, our analysis focused on surrogate lipid markers rather than hard cardiovascular endpoints, though ApoB and LDL-C are well-validated biomarkers of risk. Finally, data on newer agents such as pemafibrate remain limited, and further investigation is needed to define their long-term clinical utility.

Conclusions

This meta-analysis reveals significant heterogeneity in the lipid-modifying effects of fibrates. Fenofibrate demonstrates the most consistent and favorable profile, significantly reducing TG, LDL-C, and apoB levels both as monotherapy and in combination with statins. Gemfibrozil exerts a modest effect, primarily on apoB, however, its use cannot be considered in combination with statins. Pemafibrate, despite potent TG lowering, fails to improve – or may even worsen – LDL-related parameters. The findings of this meta-analysis underscore the importance of distinguishing between fibrates when considering adjunctive lipid-lowering therapy. These differences should guide the selection of fibrates in clinical practice, especially for patients with mixed dyslipidemia or residual risk despite statin therapy.

Conflict of Interest

Sining Xie, Federica Galimberti, Elena Olmastroni report no disclosures. Alberico L Catapano received research funding and/or honoraria for advisory boards, consultancy or speaker bureau from Amarin, Amgen, Amryt, AstraZeneca, Daiichi Sankyo, Esperion, Ionis Pharmaceutical, Medscape, Menarini, Merck, Novartis, Peer Voice, Pfizer, Recordati, Regeneron, Sandoz, Sanofi, The Corpus, Ultragenyx, Viatris. Manuela Casula received honoraria for speaker bureau from Sobi and Ultragenyx.

Author contributions

Sining Xie and Manuela Casula made the contributions to the concept and design. Sining Xie and Federica Galimberti were responsible for the acquisition, and interpretation of data. Sining Xie and Elena Olmastroni did the statistical analysis. Sining Xie and Elena Olmastroni prepared the draft of the manuscript. All authors contributed to the critical revision of the manuscript. Alberico L. Catapano provided overall supervision of the study.

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The X Spring Meeting of Young Researchers of the Italian Society of Diabetology (SID), the Italian Society of Geriatrics and Gerontology (SIGG), the Italian Society of Arterial Hypertension (SIIA), the Italian Society of Internal Medicine (SIMI), the Italian Society of Cardiovascular Prevention (SIPREC) and the Italian Society for the Study of Atherosclerosis (SISA)

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CONFERENCE REPORT

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The X Spring Meeting of Young Researchers, jointly organized by the Italian Society of Diabetology (SID), the Italian Society of Geriatrics and Gerontology (SIGG), the Italian Society of Arterial Hypertension (SIIA), the Italian Society of Internal Medicine (SIMI), the Italian Society of Cardiovascular Prevention (SIPREC), and the Italian Society for the Study of Atherosclerosis (SISA), took place in Rimini from April 6 to 8, 2025. This year's event, titled *"Spring MeeTENng – Between the PAST and FUTURE of Research, Spring is always PRESENT"*, was coordinated by young researchers from the aforementioned scientific societies, with SIGG joining for the first time. The Congress featured five thematic sessions, each addressing timely issues in the prevention and treatment of cardiometabolic diseases, offering a retrospective and forward-looking perspective spanning the past decade.

The Congress was organized by "young researchers for young researchers" from the scientific societies listed first and operating in the cardiometabolic field. The Congress hosted five different sessions, each addressing crucial issues in the prevention and treatment of cardiometabolic diseases, with a perspective that spanned the past ten years and looked toward future developments. More than 100 young researchers actively participated in oral and poster sessions, presenting and exchanging ideas on their latest findings. In the following report, we outline the core themes explored in the Meeting's lecture program.

The Meeting opened with a session addressing "non-traditional" cardiovascular risk factors. Among these, Luca D'Onofrio discussed the cardiovascular implications of autoimmune diabetes, particularly Type 1 Diabetes (T1D) and Latent Autoimmune Diabetes in Adults (LADA). It is well known that T1D is characterised by progressive β-cell destruction [1, 2], with an early onset. However, the incidence of T1D is the same before and after the age of 30 and people with T1D at the end are at increased cardiovascular mortality [3]. Life expectancy in patients with T1D is reduced by over a decade compared to the general population [3].

Subjects with T1D have a long disease duration that is one of the major risk factor for the development of cardiovascular disease (CVD) complications. Further, subjects with T1D showed several additional risk factors linked with CVD, such as poor glycemic control [4], dyslipidemia [5], hypertension [6], and heart failure [7]. D'Onofrio presented current stratification criteria for CV risk, with very high risk being defined by target organ damage or clustering of risk factors [8]. Regarding LADA, data from the UK Prospective Diabetes Study (UKPDS) [9] suggest that, although it is often misclassified as Type 2 Diabetes (T2D), individuals with LADA tend to have a lower cardiovascular risk, but the risk did not differ after adjustment for traditional cardiovascular risk factors.

The session concluded with a look toward future perspectives, emphasizing precision medicine approaches and earlier, tailored cardiovascular risk management in autoimmune diabetes [10].

The following session was dedicated to translational research in the cardiometabolic field, highlighting efforts to bridge basic science and clinical application.
Rosa Maria Bruno examined the role of vascular aging and presented the latest information on pulse wave velocity (PWV), an established biomarker for assessing cardiovascular risk and target-organ damage in individuals with hypertension. The application of PWV in clinical practice is limited due to a lack of standardization and consistency. Therefore, updated recommendations for validating devices that measure PWV were provided, along with detailed instructions for various types of devices, including their reference standards, study populations, and data analyses [11]. PWV is used to calculate vascular age, a widely recognized indicator of cardiovascular health that may correlate more closely with cardiovascular disease outcomes than chronological age [12]. The European Society of Hypertension guidelines recommend the use of carotid-femoral PWV to assess hypertension-mediated organ damage. However, its use is not widespread due to the lack of user-friendly devices. In the CARDIS Study, a novel laser Doppler vibrometer-based device for measuring carotid-femoral PWV has shown potential for development and could be used in primary healthcare settings for the early diagnosis and prevention of CVD [13].
Chiara Macchi provided an overview of the evolving role of extracellular vesicles (EVs), illustrating their transition from being regarded as mere cellular "trash bags" to being recognized as key players in translational research and promising therapeutic tools. EVs are key mediators of intercellular communication released by all cell types, from which they derive the bioactive cargo which is transported through the interstitial fluid and blood to enable communication with adjacent cells and distal tissues. Bioactive cargo encompasses a wide range of functional molecules, such as lipids, microRNAs and numerous proteins. From a technical perspective, the International Society for Extracellular Vesicles (ISEV) published the "Minimal Information for Studies of Extracellular Vesicles" (MISEV) in 2014. These guidelines have been continuously updated as a consequence of the ongoing research in this field, with the most recent version released in 2024. The goal of these new guidelines was to provide researchers with an updated snapshot of available approaches and their advantages and limitations for production, separation, and characterization of EVs from multiple sources. Overall, EVs represent an innovative tool in research for uncovering unknown molecular mechanisms. In the field of atherosclerotic burden, preclinical and clinical studies have shown that EVs can mediate the ability of proprotein convertase subtilisin kexin type 9 (PCSK9), one of the main regulator of LDL receptor, to modify their cargo, to promote a pro-inflammatory phenotype [14, 15]. Recently, EVs have gained attention for their potential as diagnostic and prognostic tools in clinical settings, since they can be used as liquid biopsies and biomarkers in cancers. The field of EVs is continuously evolving, with current research focusing on the bioengineering of EVs by loading them with exogenous cargo. This approach holds great promise for the development of engineered EVs to tailor personalized medicine.
The second day of the Meeting commenced with a session entitled "PCSK9: From Myth to Legend." The session highlighted PCSK9 as a central theme in cardiometabolic research over the past decade. Ten years ago, the inhibition of PCSK9 was proposed as a promising therapeutic strategy; today, PCSK9 inhibitors are well-established in clinical practice, and novel approaches, including epigenome editing, are under investigation. Damiano D'Ardes provided an overview of the clinical development of PCSK9-targeted therapies with his presentation "PCSK9: A star is born".

He explained as the therapies targeting PCSK9 have represented a real revolution in the field of lipidology and medicine. All the technologies connected to therapy targeting PCSK9 were discussed, in particular parenteral drugs, such as monoclonal antibodies and siRNAs [16], but also future oral treatments currently being tested [17]. The inhibition of PCSK9 not only ensures a significative reduction of LDL-cholesterol levels but also shows better clinical outcomes, reducing cardiovascular events and mortality in different categories of patients. In particular, they have radically changed the lives of many hypercholesterolemic patients, especially those with familial hypercholesterolemia (FH) [18].

Moreover, it has been underlined that PCSK9 interacts with LDLR family receptors and it is mainly produced by the liver but it is also

produced by extrahepatic tissues being involved in a great number of activities influencing many processes such as for example inflammation, response to infection and neuronal function: consequently it seems important to continue translational and clinical research on PCSK9 which could reserve further surprises and new therapeutic horizons.

Following this, emerging strategies aimed at modulating PCSK9 expression through epigenetic interventions in FH have been discussed. While genome editing can inactivate PCSK9, it involves permanent DNA modifications and potential genotoxicity.
Martino Alfredo Cappelluti discussed reversible, non-mutagenic alternative based on epigenome editing. Transcriptional repressors (ETRs) were engineered by fusing zinc-finger proteins (ZFPs) to three epigenetic effectors: KRAB, DNMT3L, and the catalytic domain of DNMT3A [19]. In vitro screening identified ZFP-based ETRs as the most potent platform for *Pcsk9* silencing. Transient delivery of ETR mRNAs via lipid nanoparticles (LNPs) to the mouse liver resulted in approximately a 50% reduction in circulating PCSK9, sustained for nearly one year. Silencing persisted even after partial hepatectomy, demonstrating the mitotic stability of the induced epigenetic marks. To simplify delivery, an all-in-one construct, EvoETR, was developed by combining all three effectors into a single ZFP fusion protein. EvoETR-8 emerged as the most effective variant, achieving approximately 75% reduction in PCSK9 levels, accompanied by enhanced DNA methylation at the *Pcsk9* promoter and minimal off-target effects, as confirmed by transcriptomic and methylomic analyses.

These results establish a "hit-and-run" epigenome editing strategy capable of inducing durable gene silencing in vivo following a single transient treatment. This platform offers a safer and reversible alternative to genome editing, holding promise for therapeutic gene regulation without permanent genetic modifications [20].
The second day concluded with Session 4, titled "Beyond Gender: Handle with Care." The session addressed the importance of considering gender/sex-related differences in both basic and clinical research. Federica Moscucci opened the session by discussing cardiovascular risk factors in the maternal-fetal dyad, highlighting how early-life exposures may influence long-term cardiometabolic health. Offspring' cardiovascular health can be affected by maternal cardiac and extracardiac environment through developmental programming, a complex mechanism consisting in the influence of maternal conditions on fetal growth [21]. Pregnancy represents a physiological stress test that can unmask underlying maternal predispositions to endothelial dysfunction, hypertension, and metabolic disorders. Conditions such as preeclampsia, gestational hypertension, and gestational diabetes are not only associated with adverse perinatal outcomes but also significantly increase the long-term risk of cardiovascular disease in the mother [22]. At the same time, the intrauterine environment profoundly influences fetal development. Exposure to maternal cardiovascular or metabolic disturbances can lead to fetal programming through epigenetic and structural adaptations, predisposing the child to hypertension, insulin resistance, and early vascular dysfunction. A prompt identification and management of cardiovascular risk is advisable, planning scheduled follow-up evaluations with a comprehensive assessment of conventional and non-conventional risk factors, promoting adequate counselling on lifestyle, cardiometabolic and mental health [23].

In addition, the implementation of structured perinatal follow-up plans and a better comprehension of the role of perinatal stress on future lifelong cardiovascular risk might allow to personalize preventive strategies and should be considered as key health objectives during pregnancy and beyond [24].
Ilaria Parrotta then focused on frailty in the elderly, presenting a comprehensive overview of sex-frailty paradox. According to the 2022 World Population Prospects, the global population aged 65 and overrepresented 10% in 2022, and is expected to rise to 12% by 2030 and 16% by 2050 [25]. Europe and North America currently have the highest proportion of older adults, with projections indicating one in four people over 65 by 2050. In Italy, as of January 2024, mortality rates declined significantly, especially among those aged >80, resulting in a life expectancy of 83.1 years. Notably, 844 people aged >105 were still alive, mostly women [26].

Aging is influenced not just by time, but by various biological, psychological, and social factors. "Successful aging" refers to maintaining good physical, cognitive, and social health, while frailty involves increased vulnerability and functional decline. A large-scale prospective study showed that frailty increases with age and is more prevalent in women, despite their longer life expectancy—a phenomenon known as the "sex-frailty paradox" [27].

Biological sex influences aging through hormones, immunity, oxidative stress and epigenetics. Estrogen offers women protective effects against cardiovascular disease and cellular aging. Men have higher testosterone, which is linked to riskier behavior and increased mortality. Metabolic and mitochondrial differences between sexes also contribute to disparities in aging and frailty [28].

Psychological and socioeconomic factors-such as stress response, social support, and healthcare access-further differentiate aging outcomes between men and women [29]. The World Health Organization advocates for a shift in focus from deficits to intrinsic capacity, a concept emphasizing individuals' physical and mental abilities throughout life. Tailored, sex-specific interventions can promote healthier, longer lives by preserving this capacity [30].
The final day of the Meeting began with Session 5, titled "Fighting Obesity on Multiple Fronts: From Adipose Tissue to Muscle." The session explored both basic and clinical research perspectives on obesity, with particular attention to its impact on muscle health and the emerging therapeutic strategies. The first lecture by Riccardo Calvani focused on a comprehensive overview of sarcopenic obesity in older adults, highlighting insights from the Sarcopenia and Physical fRailty IN older people: multi-componenT Treatment strategies (SPRINTT) project aimed at promoting healthy aging.

Due to demographic transition and rising obesity rates, a large proportion of older adults worldwide suffer from sarcopenic obesity, which significantly increases the risk of adverse health outcomes, and affects overall quality of life [31]. The pathophysiology of sarcopenic obesity is rooted on the synergistic effects of excess adiposity with age-related neuromuscolar changes. The supraphysiological decline of muscle strength and mass that defines sarcopenia is mainly due to the loss of type II (fast-twitch) fibers, which are responsible for generating muscle strength and power [32]. In the presence of obesity, both systemic (i.e., hormonal imbalances), and local factors, such as increased intra- and intermuscular fat, exacerbate age-related muscle decay. Alterations in the biological "hallmarks of ageing", such as chronic inflammation, satellite cells depletion, mitochondrial dysfunction, dysbiosis, cellular senescence and impaired macroautophagy, may further promote fat deposition and loss of lean mass and strength [33]. Lifestyle modifications, including calorie restriction with adequate protein intake and exercise (combining resistance and aerobic routines) are the cornerstone interventions to counteract sarcopenic obesity in older adults. In the SPRINTT trial, a multicomponent intervention (combining exercise with personalized nutritional counseling) reduced the risk of developing mobility disability over a follow-up of 28 months in older adults with physical frailty and sarcopenia (mean age 79.3 years, 37% BMI>30 Kg/m^2) [34]. Participants enrolled in the multicomponent intervention group also demonstrat-

ed a significant preservation of muscle mass compared to control group. Emerging pharmacological strategies, including glucagon-like peptide-1 (GLP-1) receptor agonists and novel geroprotectors, show promise. However, the potential impact on muscle mass remains a significant concern that warrants further investigation.

The session continued with Carla Greco, who addressed new pathophysiological bases of obesity and the ongoing pharmacological revolution reshaping its management.

Obesity is a chronic relapsing progressive disease process characterised by excess adiposity that impairs health and affects about 650 million people worldwide [35, 36]. It increases the risk for multiple metabolic complications (type 2 diabetes, metabolic dysfunction associated steatotic liver disease, cardiovascular disease as well as many, osteoarthritis, obstructive sleep apnoea) and also has serious social and psychological consequences, such as low self-esteem and clinical depression [37]. Lifestyle interventions (diet, exercise and behavioural changes) are the cornerstone of obesity management resulting in up to 10% mean weight loss. Further, metabolic-bariatric surgery represents an efficacy option for treatment of obesity disease. Indeed, bariatric procedures allow a mean weight loss of 25-30%. Considering the pharmacological approach, since the beginning of the 19th century a variety of drugs have been evaluated to decrease body weight and/or to improve metabolic complications of obesity: among many others, thyroid extracts, amphetamines, serotonergics and lipase inhibitors [38]. These molecules have represented for a long time the clinician's armamentarium for weight management, but with suboptimal efficacy and high burden of adverse events [38]. Today, emerging alternatives of novel agents and combinations populate the current obesity therapeutic pipeline [39]. In particular, efforts have been directed toward developing incretin-based pharmacotherapies for treating obesity. The incretins are the peptide hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), which are secreted from the gut following nutrient intake [40] and involved in the pathophysiology of obesity [41]. Therefore, in the last decade, GLP1 receptor agonists (liraglutide and semaglutide) and GLP1/GIP dual agonist (tirzepatide) have been approved as a pharmacological weight management tool in subjects with complicated-overweight or obesity, in addition to lifestyle change. Briefly, these drugs induce anorectic effects through activation of receptors located in the central nervous system with reduction of appetite resulting in decreased energy intake [42]. Moreover, the molecules also determine effects on gastric motility which promotes satiety and, for tirzepatide a direct effect on adipose tissue has been demonstrated [43]. The efficacy of incretin-based drugs allows reaching up to 22-24% of body weight, making it competitive with bariatric surgery.

In conclusion, nowadays the US Food and Drug Administration has approved 6 agents for chronic weight management in individuals living with complicated-overweight and/or obesity, including orlistat, phentermine-topiramate, naltrexone-bupropion, liraglutide, semaglutide, and recently tirzepatide.

The Meeting, following tradition, concluded with an unconventional session that this time was dedicated to a debate about denialism, featuring interventions by Fabrizio Elia and Giovanni Talerico. Through an engaging and thought-provoking discussion, the workshop explored the cognitive, social, and emotional roots of false beliefs, scientific denialism, and conspiracy thinking. The session provided participants with critical tools to better understand—and counter—misinformation and pseudoscience.

This final session was a fitting conclusion to a Meeting that not only offered an in-depth update on cardiometabolic research but also emphasized the broader responsibility of scientists in communicating evidence-based knowledge with clarity and integrity.

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SELECTED ABSTRACTS

Congress Abstract, Spring Meeting 2025, Young Researchers

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Phenoage and phenoageaccel do not outperform chronological age in predicting physical function decline and mortality in community-dwelling older adults

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Aim: Chronological age is a strong predictor of physical decline and mortality but fails to capture inter-individual variability in health and aging. Biological age metrics, such as PhenoAge and PhenoAgeAccel, integrate clinical biomarkers (e.g., inflammatory markers) to better reflect physiological aging. While these measures may predict well inflammation-driven outcomes, their utility for broader functional outcomes in general older populations remains unclear. This study compares their predictive power for physical function decline and all-cause mortality to chronological age in community-dwelling older adults.

Methods: Data from the InCHIANTI study were analyzed[3]. Participants aged ≥65 years with complete biomarker data for PhenoAge calculation (creatinine, albumin, glucose, C-reactive protein, red cell distribution width, mean corpuscular volume, lymphocyte percentage, white blood cell count, and alkaline phosphatase) and baseline Short Physical Performance Battery (SPPB) assessments were included (N=979; median age 73 years; 56% women). PhenoAgeAccel was calculated as the difference berween PhenoAge and chronological age. Physical function was assessed using the Short Physical Performance Battery (SPPB). Linear mixed models and Cox regression assessed associations with longitudinal changes in a continuous rescaled score of SPPB (rSPPB) and 10-year all-cause mortality. Logistic regression examined, in a subset of participants with normal physical function at baseline (N=504) the associations of these metrics with the onset of compromised physical function, defined as a drop in SPPB score from normal (≥10) at baseline to impaired (<10) at 6-year follow-up.

Results: Chronological age showed the strongest association with rSPPB decline (-0.50 points/10 years, p<0.001) and all-cause mortality (HR 1.15, p<0.001). PhenoAge and PhenoAgeAccel were associ-

ated with physical function decline (-0.32 and -0.15 points/10 years, respectively; p<0.001) and mortality (HR 1.10 and 1.09, respectively; p<0.001) but did not outperform chronological age. For the onset of compromised physical performance, chronological age demonstrated the strongest association and the highest predictive accuracy (OR 1.17, AUC=0.71) compared to PhenoAge (OR 1.10, AUC=0.69) and PhenoAgeAccel (OR 1.05, AUC=0.55).

Conclusions: While significantly associated with physical function decline and mortality, PhenoAge and PhenoAgeAccel do not surpass chronological age as predictive tools for these outcomes in general older populations. These outcomes are likely influenced by a complex interplay of factors – including musculoskeletal, psychosocial, and environmental determinants – that extend beyond those captured by these measures biomarker panels. These findings highlight the need for more comprehensive biological aging metrics to improve risk stratification and intervention planning in geriatric populations.

Circulating mitochondrial DNA signature in cardiometabolic patients

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Aim: Circulating mitochondrial DNA (mtDNA) profiles could refine risk stratification, but current methods do not account for different fractions of circulating mtDNA. We aimed to explore whether patients with cardiometabolic disease have a specific signature of the total circulating mtDNA profile.

Methods: We performed a complete clinical assessment, including blood tests, 12-lead ECG and ultrasound at rest and during cardiopulmonary exercise. Ultrasound congestion was defined at rest as inferior vena cava of ≥21 mm, lung B-lines ≥4, or discontinuous renal venous flow. In fasting whole blood and plasma samples collected at rest, we simultaneously measured the copy number of the cellular and cell-free components of mtDNA by real-time quantitative polymerase chain reaction. We calculated the ratio of cell mtDNA to cell-free mtDNA as an index of mitochondrial efficiency.

Results: We enrolled 120 consecutive patients: 42% with HF and preserved ejection fraction (HFpEF), 33% with HF and reduced ejection fraction (HFrEF) and 25% at risk of developing HF; 35% had diabetes. Cell-free mtDNA was increased in patients with HF (and higher in HFrEF than HFpEF) and with diabetes. Cell-free mtDNA was higher in patients with systemic inflammation (high-sensitivity C-reactive protein [hs-CRP] ≥0.2 mg/dL with neutrophil-lymphocyte ratio [NLR] >3) and more ultrasound signs of congestion. The mtDNA ratio showed opposite trends (all p<0.05). Cell-free mtDNA and mtDNA ratio independently predicted the presence of ≥2 ultrasound signs of congestion and effort intolerance (peak oxygen consumption <16 mL/kg/min) at ROC analysis and using multivariable regressions after adjustment for age, sex, hs-CRP, NLR, high-sensitivity Troponin T and NT-proBNP.

Conclusions: Cardiometabolic patients have an altered circulating mtDNA signature characterised by higher cell-free mtDNA and lower mtDNA ratio. Both are associated with impaired response to exercise, higher systemic inflammation and increased congestion. Circulating mitochondrial profile could be a new biomarker of mitochondrial status in cardiometabolic diseases.

Evaluations of metabolic and innate immunity profiles in subjects with familial hypercholesterolemia with or without subclinical atherosclerosis

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Aim: Familial hypercholesterolemia (FH) is a genetic condition characterized by high low-density lipoprotein cholesterol (LDL-C). The presence of risk modifiers could promote the atherosclerotic injury beyond LDLC. Our aim was to evaluate metabolic and innate immunity profiles in FH subjects with or without subclinical atherosclerosis.
Methods: In this cross-sectional observational study, we evaluated 211 genetically confirmed FH subjects on LDLC target and without cardiovascular diseases. Biochemical analyses, LDL-C burden (LCB) calculation and vascular profile evaluation were obtained from all subjects. Study population was divided into two groups according to subclinical atherosclerosis: the subclinical atherosclerosis (SA) group and non-subclinical atherosclerosis (NSA) group.
Results: SA group had higher LDL-C at diagnosis (288.35±24.52 vs 267.92±23.86, p<0.05) and LCB (13,465.84±3617.46 vs 10,872.63 ±3594.7, p<0.001) than NSA group. SA group had higher white blood cell count (WBCC, 6.9±1.66 vs 6.1±1.16), neutrophil count (NC, 4.2±1.3 vs 3.6±1.11), monocyte count (MC, 0.8±0.2 vs 0.4±0.1), triglyceride to high-density lipoprotein ratio (TG/HDL, 1.73±0.72 vs 1.45±0.69), triglyceride-glucose index (TyG, 8.29±0.35 vs 8.01±0.33) than NSA group (p value for all <0.01). Multivariate logistic regression analysis showed that LCB (p<0.01), WBCC (p<0.01), NC (p<0.05), MC (p<0.05) were associated with subclinical atherosclerosis. Simple linear regression analyses showed that LCB was associated with WBCC, NC, MC (p value for all <0.01).
Conclusion: An increased LCB and an impaired innate immunity profile were found in FH subjects with subclinical atherosclerosis and they were independently associated with atherosclerotic injury. LCB could modulate the innate immunity profile.

Role of multidisciplinary approach with nutritional counseling in MASLD patients on fibrosis and metabolic parameters

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Background and aims: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is a chronic liver condition affecting approximately 30% of the global population. The burden of MASLD is significantly impacted by the rising prevalence of obesity and type 2 diabetes (T2DM). There is no specific medical therapy approved and last guidelines (2024) primarily focus on lifestyle modifications and nutritional interventions. Aims of our study are to assess the effectiveness of a multidisciplinary hepatological and nutritional approach in adult patients with MASLD, focusing on changes in key anthropometric parameters such as weight, BMI, waist circumference and fibroscan value.
Patients and Methods: We enrolled 52 consecutive patients, affiliated to MASLD multidisciplinary Clinic, Liver Unit Verona. Patients were enrolled between January 2022 and September 2024. All patients underwent a hepatological and internistic visit followed by a nutritional evaluation at baseline. Patients were evaluated based on recent laboratory tests and an initial non-invasive assessment of liver fibrosis using TE with FibroScan (Echosens). The follow-up process included one or more reassessments, both hepatological and nutritional. In particular, the hepatological follow up consists in a 6-month follow up visit for a reassessment of blood tests and TE, the dietician follow-up usually consists on a 3-month evaluation in order to obtain a better compliance to the dietetic plan.
Results: All 52 patients, median age of 53 years (IQR 13.3), male 69%, underwent after hepatological visit to nutritional approach. At the baseline visit, pharmacological treatment for arterial hypertension was optimized in 26% of patients with hypertension. Lipid lowering therapy was introduced or optimized in 60% of dyslipidemic patients. Antidiabetic therapy was adjusted in 43% of patients with T2DM. TE showed a median liver stiffness of 6.10 KPa (IQR 2,75 KPa) and a median controlled attenuation parameter (CAP) value of 282 dB/m (IQR 71 dB/m). After 6 months of follow up, we observed a median reduction of 2 Kg of body wight with a significant improvement (p<0.05) in the lipid profile with reduction in total cholesterol levels (median values: 194 to 172 mg/dL), in LDL levels (122 to 102 mg/dL), in triglyceride levels (143 to 120 mg/dL), and an increase in HDL levels (46 to 47 mg/dL at follow-up). We observed a slight decrease in HbA1c (41 to 40 mmol/mol at follow-up, p=0.07). No significant changes were found in the hepatic profile, TE at follow u 5.10 KPa (IQR 2,55 KPa) and CAP 280 dB/m (IQR 64 dB/m). Only in subgroup with higher TE value at baseline (upper third quartile) we observed a significant reduction (p<0.05).
Conclusion: The results of our study highlight that multidisciplinary approach with nutritional intervention, among patients with MASLD, reached a better biochemical profile and for those with a significant levels of liver stiffness at also an improvement in FibroScan value.

Clinical characteristics and genetic predisposition of dyslipidemic patients with statin intolerance

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Background: Statin therapy represents the gold standard in lipid lowering therapy, although it is associated with an increasing rate of therapeutic abandonment especially due to the onset of muscle symptoms (statin associated muscle symptoms SAMS). In literature, a higher incidence of SAMS in the female population has already been documented, probably attributable to differences in pharmacokinetics and pharmacodynamics between genders. A relevant element in this condition would seem to reside in the SLCO1B1 gene, responsible for the tissue transport of statins, whose mutation would determine an increased plasma concentration of the same with consequent development of SAMS.
Aim: The aim of our study was to evaluate the real-life prevalence of statin intolerance in patients referred at our Center and how this determines whether or not the 2019 ESC guideline's LDL target was reached. The influence of genetic factors (specifically the SLCO1B1 mutation) and of patients' general and clinical characteristics (gender, age, BMI) on the achievement of the target was also analyzed.
Methods: We selected a population of 185 patients attending our Center and enrolled in the LIPIGEN project (96 F; 89 M), of whom 131 FH+ (67 F; 64 M). In 97% of the total population it was possible to evaluate the SLCO1B1 gene, which was found to be mutated in 67 out of 179 patients (37 F; 30 M). The mean age of our patients was 35 years (18 to 74 years; 41 f, 30 m), the mean BMI was 24.2 (23.9 f; 24.5 m).
Discussion: In accordance with the literature, our data showed a greater statin intolerance in female (58% f vs 42% m). In particular, Atorvastatin was the worst tolerated, with predominantly SAMS development even in the absence of CPK elevation (only 2 patients). If intolerance was referred by the patient, we preferred to shift to Rosuvastatin, generally characterized by better tolerability.
Intolerance showed a continuous growth trend in relation to age in both sexes, more significant in female (5-fold increase from 35 to 75 years).
The evaluation of the BMI was affected by the different numerical representation between classes, given the prevalence of normal weight and overweight population. From our preliminary data, the BMI would seem to be directly correlated with the development of statin intolerance; less significant the correlation with the achievement of the target since the poor representation of some groups could determine confounding results.
Of the 179 patients analyzed for mutations of the SLCO1B1 gene, 37% presented its mutation. By stratifying the data based on sex, the influence of this mutation on the development of statin intolerance in female was confirmed, independently of the diagnosis of familial hypercholesterolemia, particularly in female FH+.
Conclusions: Statin intolerance still represents an obstacle to therapeutic compliance and the achievement of the LDL target. Particular importance seems to be related to age and the presence of SLCO1B1 mutations; the role of BMI/waist circumference is still uncertain.

Short-term cognitive and functional decline in older patients undergoing elective cardiac surgery: preliminary results of a longitudinal study

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Aim: The implementation of new techniques has significantly reduced mortality and morbidity in older cardiovascular patients undergoing cardiac surgery. However, few studies have assessed post-surgery functional and cognitive outcomes in this demographic. This study aims to characterize baseline cognitive and functional status of patients undergoing elective cardiothoracic surgery (CS) and to evaluate their modifications over a 3-month follow-up.
Methods: Data were extracted from a prospective study starting May 2023. Patients aged 65+ undergoing elective CS received pre-operative geriatric assessments. Postoperative complications and delirium were monitored, with a 3-month follow-up assessing functional and cognitive status. Kruskal-Wallis and Fisher's tests analyzed clinical and demographic features, and linear regression examined the relationship between follow-up functional autonomy and preoperative cognitive status.
Results: Seventy-seven patients (median age: 72 [IQR, 68.00-75.25], 53% males) were included in the study, showing a median Instrumental Activities of Daily Living (IADL) of 8 [5.00-8.00]. The median pre-operative Montreal Cognitive Assessment (MoCA) was 22.88 [19.96-24.80]. 21.4% of patients with impaired cognitive performance at baseline experienced delirium, compared to 2.3% of those without impairment (p=0.052). At follow-up, 58 patients were re-assessed, showing a median IADL of 7.5 [5.00, 8.00] and an almost 64.3% prevalence of impaired cognitive performance on Telephone MoCA testing. At the linear regression analysis, pre-operative MoCA was correlated with three-month IADL decline [β = 0.144; 95% CI 0.044-0.243; p=0.005].
Conclusions: Routine cognitive assessments reveal significant cognitive deficits in older CS patients. Preliminary findings suggest a link between preoperative cognitive impairment and functional decline.

In vivo MTD study of new potential inhibitors of Proprotein Convertase Subtilisin/Kexin 9 (PCSK9)

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Aim: This study aims to determine the *in vivo* safety of newly synthesized PCSK9 inhibitors (MR-compounds). PCSK9 controls peripheral and central cholesterol levels and it plays a crucial role in hypercholesterolemia, being a well-established pharmacological target to treat this pathology, while a possible involvement in the aetiopathogenesis of AD has been postulated. Currently, the available PCSK9i to counteract hypercholesterolemia are very expensive biotechnological drugs and only subcutaneously administrated. Based on these premises, orally bioavailable small-molecules may be a valuable addition to existing treatments.
Methods: After preliminary *in vitro* screening in human hepatocyte and neuroblastoma cells of 30 compounds, 4 compounds were selected to test tolerability and bioavailability *in vivo* in wild-type mice (C57BL/6J) at 12.5mg/kg, 25mg/kg, 50mg/kg, and 100mg/kg for 5 days. MR-532 and MR-533 were administered subcutaneously, while MR-3 and MR-644 both subcutaneously and orally. Body weight and phenotype analysis were assessed daily to evaluate tolerability and macroscopic toxicity. After the sacrifice, hepatic toxicity (histological analysis and ALT activity) and biodistribution (LC-MS/MS) were evaluated.
Results: All doses of compounds were well tolerated (no changes in body weight, food intake, coat; no lethargy was observed). The MR-532 and MR-533 at 100mg/kg did not show elevated levels of ALT activity compared to vehicle (66mU±55, 76mU±127, and 130mU±203, respectively) or inflammatory cell infiltration or necrosis in liver sections (histological analysis). Interestingly, MR-532 and MR-533 were detected at all doses in plasma (261-318nM; 159-192nM), liver (522-1063pmol/g; 2824-3135mol/g) and brain (513-779pmol/g; 457-380mol/g), respectively, without a dose-dependent trend. MR-3 and MR-644 analyses are in progress.
Conclusion: All tested compounds proved to be safe. MR-532 and MR-533 showed plasma and hepatic bioavailability. They can reach the CNS, although at low concentrations. Further investigations are needed to understand how the route of administration affects biodistribution and to evaluate the efficacy of these compounds in cardiovascular and neurodegenerative diseases.

Clinical outcomes of early post-discharge cardio-geriatric ambulatory care in frail patients after acute heart failure. A controlled before-and-after study

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Aim: To assess whether an early post-discharge Cardio-Geriatric (CG) outpatient service reduces 1-year mortality compared to usual care (UC), and to evaluate 1-year rehospitalization rates and days alive and out of hospital (DAOH).
Methods: In this single-center, controlled before-and-after study, patients aged ≥75 years hospitalized for acute HF were included. In the UC group, patients discharged between January 2018 and December 2019 received standard follow-up with referrals to a cardiologist and general practitioner. In the CG group, patients discharged between January 2020 and December 2022 attended a Cardio-Geriatric ambulatory care within three weeks of discharge. Primary outcomes were one-year all-cause mortality, heart failure readmissions, and days out of hospital (DOAH). The effectiveness of CG follow-up was assessed using a 1:1 propensity score matched (PSM) analysis.
Results: A total of 652 patients (mean age 86 years, 56% female) were included in the study, with 477 receiving UC and 175 referred to CG follow-up. After propensity score matching of 350 patients (50% CG), we observed a significant reduction in 1-year rehospitalizations (36.5% vs. 50.8%, p<0.001) and mortality (20.0% vs. 40.0%, p<0.001) in the CG group. CG patients also had nearly double the days alive and out of hospital (DAOH) compared to UC patients (300±100 vs. 162±145 days, p<0.001). Cox regression analysis confirmed that the CG integrated approach was a protective factor for mortality [HR 0.34, 95% CI: 0.24-0.47]. Respiratory diseases, neurological conditions, and infections were common causes of readmission.
Conclusion: Early referral to a CG outpatients service post-discharge for acute HF significantly improves outcomes, highlighting the value of integrated care for older adults with complex needs.

Low high-density lipoprotein cholesterol, but not high low-density lipoprotein cholesterol, associates with systemic metabolic alterations

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Aim: Dyslipidemia encompasses various forms of lipid abnormalities and represents a central component of metabolic syndrome. The relationship between dyslipidemia subtypes and broader metabolic profiles is poorly characterized in modern populations. This study provides a comprehensive metabolic characterization of patients presenting with distinct dyslipidemic patterns – low high-density lipoprotein cholesterol (HDL-c) and elevated low-density lipoprotein cholesterol (LDL-c) – at a dedicated tertiary-center outpatient clinic.
Methods: Patients evaluated at the Metabolic Health Clinic of San Raffaele Hospital, Milan, between January 2023 and October 2024, were included. Medical history, anthropometrics (i.e. body mass index, BMI, and waist circumference), serum lipids and liver enzymes were recorded. Patients with low HDL-c or high LDL-c, as defined according to current guidelines, were compared to patients with normal values. Network analysis identified patient distinct metabolic clusters.
Results: A total of 496 individuals were included. Patients with low HDL-c levels (n=193, 38.9%) exhibited higher BMI (28.6 vs 25.6 kg/m^2, p<0.001), waist circumference (100.0 vs 94.0 cm, p<0.001), ALT levels (28.0 vs. 23.0 U/L, p<0.001), and triglycerides (146.0 vs. 99.0 mg/dL, p<0.001), and a greater prevalence of fatty liver disease (33% vs. 21%, p 0.006) and arterial hypertension (51% vs. 39%, p 0.012) than those with normal HDL-c levels. HDL-c showed significant inverse correlations with both BMI (R coefficient -0.272, p<0.0001) and waist circumference (R coefficient -0.325, p<0.0001). Network analysis highlighted strong associations among HDL-c, triglycerides, ALT levels, BMI, and waist circumference. Conversely, high LDL-c levels, found in 382 (77%) patients, showed no association with metabolic parameters.
Conclusions: Low HDL-c was associated with obesity, central adiposity, hypertriglyceridemia, and fatty liver disease. In striking contrast, LDL-c appears to be independent of these metabolic alterations. These findings underscore the interconnectedness of HDL-c with the metabolic landscape, while emphasizing the importance of assessing LDL-c levels regardless of patient anthropometrics and metabolic phenotype.

Single nucleotide polymorphisms (SNPs) in patients with acute ischemic stroke: A prospective study of the relationship between genetic, acute phase cytokine levels and stroke prognosis

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The genetic basis of complex diseases like ischemic stroke probably consists of several predisposing risk factors, such as genes involved in inflammation and thrombotic pathways. Some genetic polymorphisms have been associated with the risk of stroke.
Aim: On this basis the aim of this study was to evaluate:
- the frequency of some single nucleotide polymorphisms (SNPs) of genes of pro-inflammatory cytokines and coagulation factors in stroke patients;
- the relationship between each identified SNP and TOAST stroke subtype;
- the relationship between the serum levels of the cytokines analyzed and the diagnostic subtype of ischemic stroke;
- the relationship between the serum levels of the analyzed cytokines and stroke prognosis regarding event recurrence, AMI recurrence, and mortality.

Materials and methods: All patients aged > 18 years admitted for acute ischemic stroke in the period between 2011 and 2021 were prospectively enrolled. Each patient was subjected to genetic analysis to evaluate various genetic polymorphisms and to the analysis of the levels of cytokines circulating in the different collection times (T0, T1, and T2). Three different biallelic polymorphisms, of the IL-10 gene were identified.
Results: 624 subjects were enrolled, including 429 patients with ischemic stroke and 195 controls. Stroke subtype: 47% LAAS, 27% LAC, and 26% CEI. Regarding the immunoinflammatory variables, patients with CEI showed significantly higher levels of serum glucose and all the cytokines analyzed, compared to patients with both LAC and LAAS.
Logistic regression analysis revealed that elevated IL-10, TNF-alpha, IL-6, and IL-1beta values are predictive of LAAS and CEI subtypes, respectively. IL-10 levels were lower in patients who developed stroke during follow-up, whereas TNF-alpha, IL-1, and IL-6 levels were significantly higher in patients with recurrent stroke at follow-up, who developed a new vascular event or who experienced death during follow-up. From the analysis of the distribution of the genotypic frequencies of the polymorphisms analyzed, a statistically significant difference emerged between the cases and the controls for all the polymorphisms in the genes of pro-inflammatory cytokines, TPA and PAI-1.
These results demonstrated an association between some pro-inflammatory and prothrombotic polymorphisms and the risk of ischemic stroke.

Prioritizing medication review for older individuals: A real-world data study using administrative databases

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Aim: Optimizing drug treatments in older individuals is essential for improving health outcomes and reducing drug-related issues. However, targeting older adults for interventions is challenging and tools to identify and prioritize individuals with potentially inappropriate medications (PIMs) are lacking. This study aims to develop a prioritization algorithm for medication review, with a proof-of-concept established using Italian administrative data, and to assess the association between PIMs and all-cause hospitalization.
Methods: Eight indicators were selected:
1) medications that should be avoid in elderly,
2) drugs linked to fall risk or orthostatic hypotension,
3) drug-drug interactions,
4) Anticholinergic Cognitive Burden,
5) Sedative Load,
6) therapeutic duplicates,
7) polytherapy,
8) drugs with higher risk of adverse drug reactions.
This study focused on the first indicator. Administrative healthcare data from Local Health Units (LHUs) in Lombardy were used to identify over 65 individuals who redeemed a PIM between 2015 and 2018, with index date defined as the first PIM redemption. Risk-set matching was used to select controls, adjusted for high-dimensional propensity scores (HDPS) logistic regression models were used to assess the odds of all-cause hospitalization within 90 days.
Results: A total of 499,511 over 65 adults across the LHUs were evaluated. Between 27.4% and 37.7% individuals were exposed to at least one PIM with higher prevalence in adults aged 65-74 years and women. After matching, 128,063 pairs were analyzed. Hospitalization rates were higher among exposed individuals (8.3-10.2%) compared to controls (5.1-6.0%). Multivariate regression showed a 55% increased risk of hospitalization for those exposed to PIMs (OR 1.55, 95% CI 1.48-1.62).
Conclusions: This proof-of-concept study made it possible to develop an analytical model, which will be implemented for the other indicators. The strength of the association between each indicator and the risk of hospitalisation will be used as a weight in the construction of the prioritisation algorithm.

Cardiovascular risk stratification in patients with inflammatory bowel disease: The role of non-invasive imaging techniques and traditional risk scores

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Background: Patients with chronic inflammatory diseases, including inflammatory bowel diseases (IBD), have a 20% increased risk for atherosclerotic cardiovascular disease (ASCVD) and death as compared to non-inflamed subjects. A more in-depth screening of patients has become important with the EMA warning for JAK-inhibitors. The current validated cardiovascular risk (CVR) stratification algorithms are based on traditional risk factors, not taking into account the contribution of chronic inflammation.
Aim of the study: Our study aimed to stratify the CV risk of IBD patients using validated scores (SCORE2/SCORE2-OP/SCORE- 2Diabetes) and performing carotid ultrasonography to identify subclinical atherosclerosis.
Materials and methods: Data from 120 consecutive IBD patients [Ulcerative Colitis(UC): 67; Crohn's disease(CD):53] aged ≥40 years under care in the IBD Unit of the University Hospital of Messina (April-to-July 2024) were collected. We recorded data on age, gender, region of origin, body mass index, smoking history, family, personal and pharmacological history, blood pressure values, biochemistry (creatinine, fasting glucose, glycated hemoglobin, total cholesterol, HDL-cholesterol, triglycerides). LDL-C/non-HDL-C were thus calculated. Additional IBD-related parameters potentially associated with an increased CVR were investigated (i.e., disease activity, current therapies, duration of disease, and extraintestinal manifestations).
Results: Based on their medical history, 48% of patients were classified as at intermediate CVR, 34% as high CVR, and 18% as very high CVR. Carotid ultrasound detected subclinical atherosclerosis in 48.3% of patients. CV risk reclassification occurred in 21%, increasing the proportion of patients with high/very-high risk from 50% to 71%. Active disease (p=0.047) and concomitant spondyloarthropathies (p=0.03) were identified as additional risk factors.
Conclusions: Our findings demonstrate that carotid ultrasonography significantly reclassifies CV risk, revealing that traditional risk scores underestimate CV risk in IBD patients. Tailored CV risk stratification, incorporating chronic inflammation, is crucial before initiating therapies like JAK-inhibitors to minimize side effects, including CV complications. Active intestinal disease and spondyloarthritis further exacerbate CV risk, underscoring the need for integrated screening and management strategies in this population.

Novel biomarkers in acute kidney injury: the role of L-FABP, CYR61, TIMP-2, IGFBP-7, PENK e KIM-1 in the diagnosis of kidney dysfunction etiology and their predictive role of structural renal damage severity

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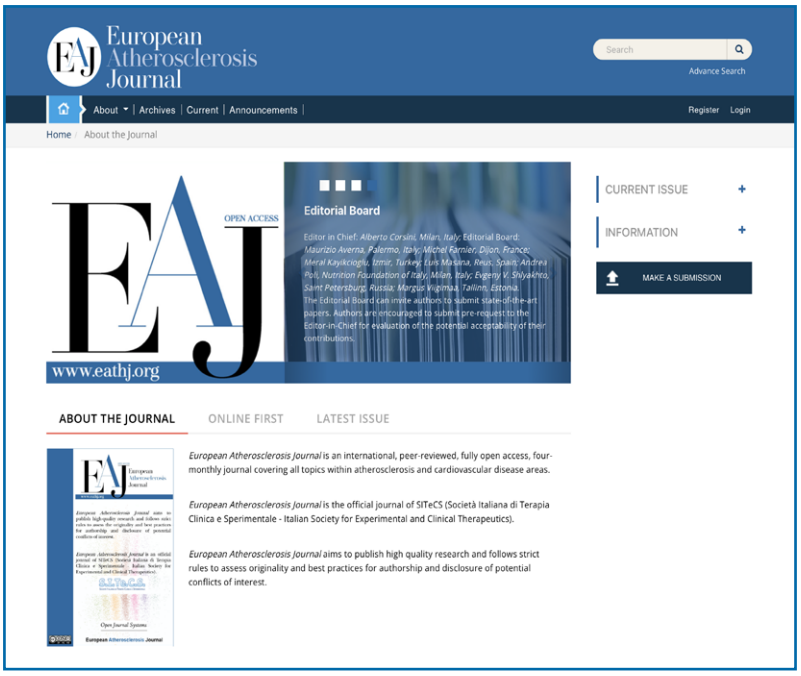
Background: Acute kidney injury (AKI) is a very common life-threatening disease. Early diagnosis is the cornerstone for limiting the progression and chronicity of renal damage and reducing mortality. Estimating the glomerular flow velocity is the most used method to evaluate renal function. However, detecting new circulating molecules has taken hold for the early identification of kidney damage. To investigate the correlation between the urinary and serum biomarker concentrations and renal dysfunction, we studied a cohort of patients with AKI, accounting for aetiology. **Aims**: Our study aimed to find an association between some new circulating markers of renal injury and the pathogenic mechanism of acute kidney failure. In particular, we have evaluated the possible association between the urinary and serum concentrations of LFABP, CYR61, TIMP2, IGFBP7, PENK, and KIM-1 and AKI's prerenal or intrinsic pathogenesis. The secondary aims of the study were (i) evaluating the possible association between the urinary or serum concentrations of the markers with the severity of the acute kidney injury by the estimation of the variation between the serum creatinine at the admission compared with the basal values reported on the previous documentation exhibited; (ii) identifying the prognostic role of these markers and evaluating their association with the range of variation of the creatinine at discharge versus the values at admission.

Methods: In this cross-sectional, observational trial, 57 patients with acute kidney disease were consecutively enrolled and underwent a complete medical history to evaluate comorbidities, physical examination, and routine blood tests after eight hours of fasting; urinary and serum concentrations L-FABP, CYR61, TIMP-2, IGFBP-7, and PENK E KIM-1 were obtained in all patients.

Results: Urinary TIMP2, NGAL, and IGFBP7 and serum PENK values were higher in patients with AKI compared with the control group, with statistical significance. Moreover, higher concentrations of FABP1, Cyr61, TIMP-2, NGAL, IGFB7, and TIMP-2 X IGFBP-7 were found in patients with renal AKI compared with prerenal aetiology. A significant association between the urinary values of FABP1 and TIMP-2 and the serum concentrations of KIM-1 (p= 0,0001) with the variation of the creatinine values from the baseline to the values at the enrollment was found. Furthermore, a statistically significant association was found between KIM-1 and the creatinine variation at the discharge compared with the admission values.

Discussion: In this trial, we evaluated the serum and urinary concentrations of some novel biomarkers of acute kidney injury in a cohort of 57 patients diagnosed with acute renal failure, divided on the aetiology. With the primary aim of finding an association between these markers and the aetiology of the kidney injury, we demonstrated a statistically significant association between the concentrations of FABP1, Cyr61, TIMP-2, NGAL, IGFB7, and TIMP-2 X IGFBP-7 and the intrinsic aetiology of the AKI. Evaluating these "early diagnostic" biomarkers could help identify the underlying physiopathologic mechanism of the renal injury: considering the role of IGFBP-7 and TIMP2 in the cellular cycle and, in particular, in the mechanisms of cellular death, it is clear how the expression of these biomarkers is increased after a direct injury to the renal cells rather than prerenal injury: the levels of IGFBP7 persisted statistically associated with the AKI's aetiology after the multinomial regression, as not affected by other variables. Furthermore, our study has found an association between some of these biomarkers and creatinine variations. In particular, urinary FABP1, TIMP2, and serum KIM-1 levels were associated with a higher variation between the creatinine values at admission compared with the basal values, supporting a possible role of these proteins in defining the severity of renal injury. Moreover, KIM-1 concentrations were proportionally associated with the change of the creatinine values during the hospitalization, with a higher KIM-1 value as much as a higher reduction of the creatinine at recovery compared with admission: we confirm the protective role of KIM-1 in the worsening of renal dysfunction, but the constitutive expression of this protein on the tubule results in gradual fibrosis and progression towards chronic nephropathy. Considering the protective role of KIM-1, the increased values of KIM-1 may be related to a higher probability of recovery from renal dysfunction. Finally, in our study, we confirm the diagnostic role of some of these molecules. In particular, we have found that urinary values of TIMP2, NGAL, and IGFB7 and serum concentrations of PENK were statistically higher in patients with AKI compared to controls. Our study provides further evidence concerning the possible use of these novel biomarkers of AKI in clinical practice. Given their diagnostic and predictive role, these molecules could be used to identify the population at risk for the development of AKI and to identify renal injury early, even before the increase in serum creatinine or cystatin C values.



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