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Systematic review and meta-analysis deciphering the impact of fibrates on paraoxonase-1 status

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ABSTRACT

Objective: A significant residual cardiovascular risk is consistently observed in patients treated with statins. A combined treatment with fibrates reduces cardiovascular events in very high-risk patients. Because this is apparently unconnected to an improvement in lipid-related outcomes we hypothesized that the cardioprotective effects of fibrates might be associated with an improvement in paraoxonase-1 (PON1) status.

Method: The search for existing evidence, using the Medline, Scopus and Cochrane databases, was systematic and followed the PRISMA statement without restrictions on publication date. We excluded non-clinical and observational studies and we extracted data on baseline and post-treatment values of serum PON1 activity and other measurements of PON1 status.

Results: Nine studies (including 12 treatment arms) in patients with hyperlipidemia, diabetes or metabolic syndrome treated with fibrates, alone or in combination with statins, were included to synthesize results. A meta-analysis of the data using a random-effects model revealed a significant increase in serum PON1 activity following fibrate therapy (WMD: 15.64 U/L, 95% CI: 6.94, 24.34, $p<0.001$), an effect that was robust and not sensitive to any particular study. Subgroup analysis indicated differences in the effect size among types of fibrates and that PON1 alterations were associated with high-density lipoprotein cholesterol changes following fibrate therapy.

Conclusions: Results indicate a significant PON1-enhancing effect of fibrates. Whether this effect is associated with a clinical benefit, although likely, remains to be further investigated.

Keywords: cardiovascular risk; HDL; hypertriglyceridemia; inflammation; lipoprotein metabolism; oxidation

1. Introduction

The addition of fibrates to statin therapy aimed to control residual vascular risk in selected patients provides reductions in cardiovascular events. Beneficial effects are limited to individuals with high risk of cardiovascular (CV) disease and/or those with atherogenic dyslipidemia (i.e., combined hypertriglyceridemia and low plasma high-density lipoprotein cholesterol (HDL-C) concentrations) [1, 2]. The likely mechanisms for the cardioprotective effects of fibrates are, however, not restricted to the effects of these drugs in reducing plasma triglyceride and/or increasing HDL-C levels [3-5]. Interpretation from clinical trials is difficult [4-6] because data are probably modulated by changes in lifestyle factors and secondary pharmacological treatments with anti-inflammatory and antioxidant effects [7-9]. Factors considering the heterogeneous structure, different intravascular metabolism and, more importantly, biological activity of lipoproteins should be included in the clinical assessment [10, 11]. The reduction in inflammation and oxidation, under these circumstances, is likely associated with an increase in antioxidant protection by paraoxonase-1 (PON1) and significant effects in the functional complexity of circulating lipoproteins [10]. PON1 is responsible for several functional properties of HDL, including antioxidant, anti-inflammatory and homocysteine-thiolactone detoxification activities. [12] Some *in vitro* data have strongly suggested that triglycerides decrease PON1 catalytic activity and have deleterious effects on HDL biological activity [13, 14]. Hence, the effects of fibrate therapy, which corrects these alterations, might provide a theoretical basis for exploring the PON1 status in CV disease especially because these effects overlap with the action of statins and the combination of these drugs might result in efficient enhancement of PON1 status and mitigation of oxidative burden [13-15].

Our research question (i.e., whether fibrates are potential modulators of PON1) is mostly relevant in the study of the metabolic syndrome as a low-grade proinflammatory and/or pro-oxidative state associated with high serum triglyceride concentrations, low HDL-C values and high cardiovascular risk [16-18]. Moreover, nutritional and lifestyle changes indicated in the management of metabolic syndrome can regulate PON1 activity [19,20]. Current data have derived from studies of PON1 but it is important to highlight the existence of three paraoxonase genes (and proteins), which probably evolved from a common ancestor [21, 22]. In the blood, PON1 is mostly bound to HDL and the relative abundance and wide distribution of PON1 in human tissues is likely associated with HDL-related delivery of PON1 into cells [23, 24]. Of note, PON1 is an enzyme that hydrolyzes a variety of substrates and promotes the degradation of oxidized lipids in both circulating lipoproteins and cells [25-28]. The enzyme is promiscuous and the developed spectrophotometric assays are based on the enzyme's capacity to hydrolyze different substrates. The choice of substrates is mostly based on low cost and availability, and paraoxon and phenyl acetate have been traditionally used as both paraoxonase and arylesterase activity are important determinants of PON1 status [27]. Indeed, accumulating evidence from clinical research has indicated that PON1 is an important contributor to CV health and consequently have proposed therapeutic strategies targeting the molecular regulation of PON1 [6, 9, 10]. Fibrates and other peroxisome proliferator-activated receptor alpha (PPARα) agonists are potential options for the stimulation of PON1 activity [29, 30]. Specifically, there are some PPAR*α* binding sites in the *PON1* gene promoter that could directly influence serum PON1 activity and PPARα activators induce the expression of apolipoprotein AI and the ATP-binding cassette A1 (ABCA1) controlling cellular cholesterol efflux [29-32]. We have systematically

assessed and synthetized the clinical evidence, using meta-analysis, to clarify the potential role of fibrate therapy in antioxidant protection by paraoxonase.

2. Methods

2.1. Eligibility criteria, protocol and search process

The inclusion criteria were specified a priori including study designs, populations, interventions, comparisons and outcome measures. The protocol was limited to: 1) clinical trials (i.e., in humans) with concurrent control groups; 2) investigation of the impact of fibrates on PON1 status; 3) treatment duration of at least 2 weeks; and 4) the presentation of information on PON1 status at baseline and at the end of the treatment period (i.e., reported outcomes commonly accepted as meaningful to assess changes in PON1). We excluded non-clinical and observational studies and there were no geographic inclusion/exclusion criteria.

Following procedures outlined previously, as well as the PRISMA and STARLITE statements [10, 33, 34], we performed a search involving an expert chain-of-citations approach, followed by a keyword-based computerized search with no restrictions on publication date. We assumed, considering the included search terms, that English-language was sufficient to identify relevant non-English-language reports. We searched Medline (http://www.ncbi.nlm.nih.gov/pubmed) and SCOPUS (http://www.scopus.com) databases using an overlapping subsets approach and applying the following Boolean expression, which included suggested Mesh terms and wild-card terms to increase sensitivity: (fenofibrate OR bezafibrate OR ciprofibrate OR clofibrate OR gemfibrozil OR “fibrates” OR “fibrate therapy”) AND (paraoxonase OR paraoxonase-1 OR "paraoxonase 1" OR “paraoxonase1” OR PON-1 OR "PON 1" OR “PON1” OR arylesterase). We found no search results in the Cochrane database of

systematic reviews (http:/www.cochrane.org). Publication status was not a criterion and to avoid the exclusion of fugitive literature, an information retrieval specialist searched Google scholar, Dissertation Abstracts International (http://www.worldcat.org), conference proceedings (eric.ed.gov) and SIGLE (system for information on gray literature) database. Hand searching of references of the retrieved articles was performed to identify additional studies but this information was considered not relevant by the review team according to our criteria. When necessary, we contacted authors to confirm the accuracy of the data and to ensure whether preliminary results or special reports were taken from the original studies.

2.2. Statistical analysis: quantitative data synthesis

We used Comprehensive Meta-Analysis software, version 2 (Biostat, Englewood, NJ, USA), for all of the analyses [35, 36]. The standard deviations (SDs) of the mean difference were calculated using the following formula: $SD = \sqrt{[(SD_{pre\text{-}treatment})^2 + (SD_{post\text{-}treatment})^2 - (2R \times SD_{pre\text{-}treatment} \times SD_{post\text{-}treatment})]}$, assuming a correlation coefficient (R) of 0.5. In case of the reporting of standard errors of the mean (SEM), SD was estimated using the following formula: $SD = SEM \times \sqrt{n}$, where n is the number of subjects. When the studies reported outcome measurements in medians and ranges, we estimated means and SD values as described [37]. The transformed and calculated data mostly consisted of the subtraction of values of outcome measurements after treatment from the value reported at baseline. The weighted mean differences (WMDs) and 95% confidence interval (CIs) were the summary statistics expressing the effect size. In this particular study the terms “effect” and “treatment” size were considered synonymous. We assessed statistical heterogeneity using Cochrane’s Q statistic and the I^2 index. We used a random-effects model and a generic inverse variance method [38, 39] to explore and to compensate for the likely heterogeneity of studies in terms of demographic characteristics, differences in study design and

the type of fibrate employed [38 - 40]. To evaluate the influence of each study on the overall effect size, we conducted sensitivity analysis using the leave-one-out method (i.e., removing one study each time and repeating the analysis) and confirmed the stability of the overall result [41-43].

2.3.Quality assessment

A systematic assessment of bias in the included randomized controlled trials was performed using the Cochrane criteria [44]. The items used for assessment were adequacy of sequence generation, allocation concealment, blinding, addressing of dropouts (incomplete outcome data), selective outcome reporting, and other potential sources of bias. According to the Cochrane recommendations, a judgment of "yes" or "no" indicated low and high risk of bias, respectively. In case of unclear or unknown risk of bias, the study was labeled as "unclear".

2.4. Meta-regression

Random-effects meta-regression was performed using unrestricted maximum likelihood method to evaluate the association between calculated WMD in plasma paraoxonase activities with respective changes in plasma LDL-C and triglycerides concentrations.

2.5. Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, and Begg's rank correlation and Egger's weighted regression tests. Duval & Tweedie "trim and fill" and "fail-safe N" methods were used to adjust the analysis for the effects of publication bias.

3. Results

3.1 Characteristics of included studies

The search process included 621 participants from prospective, interventional studies focusing on the influence of fibrate administration, alone or in combination with statins, on PON1 activity and/or concentration in hyperlipidemia, diabetes or metabolic syndrome. Ninety-seven reports were initially identified and 20 were immediately considered non-eligible mostly by duplication or lack of relevance. The remaining 77 electronic reports were obtained and data were extracted and coded for further review and meta-analysis studies by at least two investigators. The team resolved disagreements on eligibility criteria and we finally selected nine studies with 12 treatment arms (Figure 1) [35, 45 - 52] according to predesigned multiple-item scales to rate overall study quality on the risk of selection bias, performance bias, attrition bias and detection bias. Included studies were published between 1998 and 2011 and the range of intervention period was between 2 and 3 months. Two of the studies included only men [46, 47] and the others were performed in men and women [35, 45, 48-52], whose age was between 44 and 61 years old. Combination of fibrates and statins was tested in two studies [45,46] whereas fibrates alone were used in the others [35, 47-52]. Two trials were designed as parallel-group studies, two had a crossover design, two were randomized trials and five were open-label designs. Characteristics of the included studies are detailed in tables 1 and 2. The risk of bias assessment of the included studies is summarized in Table 3.

3.2 Fibrate therapy increases serum paraoxonase-1 activity

Meta-analysis of the data revealed a significant increase in serum PON1 activity following fibrate therapy (WMD: 15.64 U/L, 95% CI: 6.94, 24.34, $p < 0.001$) (Fig. 2A). In

leave-one-out analysis (Fig. 2B), this effect was apparently robust and not sensitive to any particular study. The choice of the type of fibrate is apparently important (Fig. 3) because gemfibrozil (WMD: 17.53 U/L, 95% CI: 11.48, 23.59, $p < 0.001$; $I^2 = 27.21\%$) and fenofibrate (WMD: 16.94 U/L, 95% CI: -2.07, 35.95, $p = 0.081$; $I^2 = 85.38\%$) achieved greater increases in serum PON1 activity than bezafibrate (WMD: 7.07 U/L, 95% CI: -13.49, 27.63, $p = 0.500$; $I^2 = 93.83\%$). We also found that, in controlled trials (four treatment arms), the effect size of fibrates on serum PON1 activity is significant (WMD: 12.60 U/L, 95% CI: 1.50, 23.70, $p = 0.026$) (Supplementary Fig. 1). Heterogeneity was expected, but it seemed not to affect the main conclusion reinforcing our decision to incorporate the studies that have enough in common that makes sense to synthesize the information. Our study also clarified that the effect of fibrates was not observed (seven treatment arms) when serum arylesterase activity [2.88 U/L (95% CI: -8.93, 14.69, $p = 0.633$; $I^2 = 94.18\%$)] or the concentrations of the PON1 protein [0.97 mg/l (95% CI: -6.87, 8.82, p = 0.808; I2 = 95.71%)] were chosen to measure the main effect (Supplementary Fig. 2).

We were curious to determine whether the differences between subgroups were associated with any of the secondary outcomes. Therefore, we conducted a meta-regression analysis in a random-effect model, which indicated the lack of associations between serum PON1 activities and changes in plasma levels of LDL-cholesterol (slope: 0.176; 95% CI: -0.489, 0.842; $p = 0.604$) or triglycerides (slope: -0.052; 95% CI: -0.195, 0.091; $p = 0.477$). Contrarily, the increase in PON1 status was sensitive to an increase in HDL cholesterol (slope: 3.495; 95% CI: 0.845, 6.146; $p = 0.009$), indicating a potential association and the putative role of HDL as a delivery factor of PON1 to cells (Fig. 4).

3.3. Publication bias

Assessment of publication bias is important in meta-analyses and other quantitative reviews because it can alter the significance of major findings (i.e., null or non-significant results are published more reluctantly). Visual inspection of the funnel plot of the study precision (inverse standard error) by effect size (mean difference) did not reveal any evidence of asymmetry (Fig. 5). Values for the Begg's rank correlation test [36] (Kendall's Tau with continuity correction = -0.02, Z = 0.07, two-tailed $p = 0.945$) and Egger's linear regression test [53] (intercept = 1.48, standard error = 1.53; 95% CI = -1.93, 4.89, t = 0.969, df = 10.00, two-tailed $p = 0.355$) excluded publication bias. We identified a potentially missing study on the right side of funnel plot. This potentially missing study was imputed using trim-and-fill method, but the imputed effect size was similar to the initial estimate of WMD (17.07; 95% CI: 8.53, 25.60). We also used fail-safe number calculations (n=250) to adjust the analysis, and we found that the possibility of altering conclusions, based on the impact of fibrates on plasma PON1 activities due to publication bias, was extremely unlikely [54, 55].

4. Discussion

Fibrate therapy increases serum PON1 activity. This effect is relevant because HDL-associated PON1 activity is a major defense against LDL oxidation, and an increase in PON1 activity appears to have anti-atherogenic effects. These facts might partially explain the significant reduction in relative risk for coronary events observed in patients treated with fibrate therapy [2]. Also, the PON1-enhancing effect of fibrates found in the present meta-analysis was similar to the effect size estimated for statins (WMD: 14.92 U/L, 95% CI: 9.46, 20.37, $p<0.001$) in accordance with their recognized antioxidant effects [56]. It is worth mentioning that, in a subgroup analysis of the ACCORD trial, the combination of fenofibrate and simvastatin in patients with high plasma triglycerides and/or low HDL cholesterol induced a 31% reduction in

the relative risk of CV events [57]. Moreover, the Adult Treatment Panel III guidelines suggest considering the prescription of fibrates, in addition to statins, for these subjects [58]. The relevance is also reinforced by a recent meta-analysis, which demonstrated that low levels of PON1 activity were a risk factor associated with increased susceptibility to CV disease [59].

Our study provides the first meta-analysis assessing the effect of fibrate therapy on PON1 activity and the results were consistent. However, subgroup meta-analyses detected a certain heterogeneity providing clues for the design of future reports to yield rigorous clinical conclusions: 1) Gemfibrozil apparently has a superior effect; 2) the potential benefit was associated with an increase in HDL cholesterol; 3) the assessment of the PON1 status should likely be limited to paraoxonase activity; and 4) the relevance of the association of PON polymorphisms and CV disease is uncertain [59, 60]. PON1 activity has been reported to be a better predictor of CV disease than PON genotypes [61-63], but the need for investigations of the genetic determinants of complex diseases cannot be overstated. Moreover, two reports included in this study [47, 48] provided indicative information that the stronger effects of fibrates on paraoxonase activity, compared with those observed on arylesterase activity, might suggest a possible influence of $PON1_{192}$ isoforms. Micro-coated fenofibrate or ciprofibrate increased PON1 activity only in QQ patients with metabolic syndrome, and the effects on QR and RR were negligible. In addition, further improvement in methods to assess the actual endogenous physiological activity of the enzymes (PON1, PON2 and PON3) might provide useful information because the action of PON2 is limited to cells and the circulating activity of PON3 and the overall lactonase activity require further research [26, 27, 64]. The measurement of PON1 concentration [47, 48, 50] did not provide further insights.

The exploration of pathways related to the modulation of HDL should be included in clinical trials, and paraoxonases are important for attenuating atherosclerosis development [65-67]. The induction of higher expression of PON1 (or PON3) in macrophages is protective via the modulation of oxidative stress, and deficiencies render mice more susceptible to atherosclerosis [68-72]. Interestingly, results in this study are illustrative of findings in *in vitro* and animal models. For example, in cultured liver cells, the addition of fibrates regulated the *PON1* promoter genes, resulting in increased PON1 enzymatic activity and expression of mRNA levels (>70%). The mechanisms are uncertain but likely involve the expression of different receptors (i.e., PPARα or liver X receptor) [62]. Similarly, HDL obtained from patients in whom fibrate therapy reduced serum triglyceride concentrations and increased HDL-C levels and serum PON1 activity (>80%) increased the ability to induce cholesterol efflux from macrophages [14]. Our interpretation reinforces the assessment of PON status as an additional or alternative treatment target in prescribing medications and assessing CV risk, [73-75].

The HDL-C-raising treatment as a strategy to reduce CV events is currently under considerable debate [76-80]. Recent clinical trials, mainly the niacin-based AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes) and HPS2-THRIVE (Heart Protection Study 2: Treatment of HDL to Reduce the Incidence of Vascular Events) studies, have suggested that the measurement of HDL-C concentrations might be an inconsistent target in the prevention of CV diseases [76, 77]. In such a controversy, the lack of information in the biological function of HDL complicates proper interpretation and final conclusions. We emphasize here that, in *post-hoc* analyses, fibrate therapy has been shown to improve microvascular diseases in patients at high CV risk; a beneficial effect in which PON1 might play a central role [81-83]. Notably,

ongoing research in selective PPARα modulators could provide valuable insights for redesigning combined treatments to decrease residual CV risk [84, 85] and to manage other metabolic and non-communicable diseases [29, 32].

The pleiotropic effects of fibrates might increase the value of our research question and support the concept that the functionality of HDL is more important than the number of lipids transported by these lipoproteins (i.e., structural disorders in lipoproteins could change their functions in cholesterol transport) [86]. Notably, fibrates are sensitive to differences in the caloric content of the diet, and they play a central role in metabolism [87-90]. Mice deficient in PON1, PON2 or PON3 are susceptible to obesity, have deficient metabolism of xenobiotics, and develop inflammatory diseases, cancer, gallstone formation, liver steatosis, and mitochondrial dysfunction [15, 32, 64, 91-93]. These findings are in agreement with evidence indicating that HDLs, when functioning properly, remove cholesterol from cells and can exert anti-inflammatory, anti-thrombotic and anti-oxidant effects [94]. Perhaps it is time to propose different therapeutic strategies for complex metabolic diseases and, consequently, to suggest new *post-hoc* analyses of clinical trials [95]. In particular, the development of novel HDL- or PON-associated treatment strategies [96, 97] could introduce additional benefits for CV health and might also serve as useful additions to the existing therapies used in the management of cardiometabolic diseases [98-103].

To the best of the authors' knowledge, this study is the first systematic review and meta-analysis investigating the impact of fibrates on plasma paraoxonase and arylesterase activities. However, the present study is subject to some limitations that have to be taken into account. First, the number of controlled trials was limited, and there was not sufficient evidence for performing a meta-analysis to assess the impact of combined statin-fibrate treatment versus

fibrate monotherapy on PON1 status. Second, available studies did not assess the association between fibrate-induced alterations in plasma PON1 activities and cardiovascular outcomes. Third, assessment of PON1 changes was not among the primary aims of most of the included studies. Finally, we only included published studies in English language into the meta-analysis, and this might have caused a selection bias.

5. Concluding remarks

Circulating PON1 is bound to HDL. The data obtained in this meta-analysis indicated that fibrate therapy increases PON1 activity. This effect is relevant because the activity of PON1 modulates anti-atherogenic properties and the maintenance of the metabolic homeostasis. Clinically, considering LDL-C as the target goal, statins are the drug of choice. Nevertheless, a substantial residual CV risk persists in patients attaining desirable LDL-C levels. The concomitant use of other drugs is a feasible option, but there are no available data on CV outcomes to guide combined therapies. In this study, we indicated some significant associations between the actions of fibrate therapy and PON1 status, which are not necessarily related to the successful correction of hyperlipidemia. These results provide additional support to the notion that an improvement of the antioxidant HDL function provided by fibrates may partially account for the cardioprotective actions of fibrates in selected patients [104, 105]. Future studies should include the assessment of the potential role of PON1-enhancing effects of fibrates in reducing CV events, and whether this improvement may also suggest potential benefits of fibrate therapy in other pathological conditions associated with oxidative stress. Moreover, since a genotype-phenotype correlation has been previously shown for PON1 activity, evaluation of the impact of functional polymorphisms within the *PON1* gene, particularly Q192R, on the PON1 response to

fibrate therapy merits further investigations. Finally, the impact of selective PPARα modulators and statin/fibrate combination on PON1 status remains to be evaluated.

Abbreviation list

ABCA1: ATP-binding cassette A1

CI: Confidence interval

CV: Cardiovascular (CV)

HDL: High-density lipoprotein

HDL-c: High-density lipoprotein cholesterol

LDL-c: Low-density lipoprotein cholesterol

PON1: Paraoxonase-1

PON2: Paraoxonase-2

PON3: Paraoxonase-3

PPAR***α*:** Peroxisome proliferator-activated receptor alpha

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

SD: Standard deviation

SEM: Standard errors of the mean

SIGLE: System for information on gray literature

WMD: Weighted mean differences

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Conflict of interest

There are no conflict of interest.

Author contribution

AS and JJ established the research question and designed the study and the search strategy. All authors participated in the interpretation of results. DA and ES mainly performed quality assessment and supervised data extraction. AS and AHA were responsible for data collection and extraction. AS performed the statistical analyses. AHA prepared graphics and joint discussions. AS, JC and JJ wrote the initial manuscript, which was discussed and finally approved by all the authors.

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Figure legends

Fig. 1. Flow chart for study search and inclusion.

Fig. 2. Fibrates increase serum paraoxonase activity. (A) We used a forest plot to indicate the impact of fibrate therapy on serum PON1 activity in the included reports. (B) To evaluate the influence of each study on the overall effect size, we conducted a sensitivity analysis using the leave-one-out method (i.e., removing one study each time and repeating the analysis).

Fig. 3. Not all fibrates are equally effective. Forest plots used in a subgroup analysis indicated the different impact on PON1 status according to the type of fibrate used for treatment.

Fig. 4. **Determinants of changes in PON1 status**. Meta-regression plots of the associations between mean changes in serum paraoxonase-1 activity and the corresponding changes in the measurement of the concentration in the serum of triglycerides, cholesterol and both low-density- and high-density-lipoproteins.

Fig. 5. Absence of publication bias. Funnel plot of precision and effect size of studies examining the lack of impact of publication bias on the relationship between fibrate therapies and serum PON1 activity. Open circles represent observed published studies, and the close circle represents an imputed unpublished study.

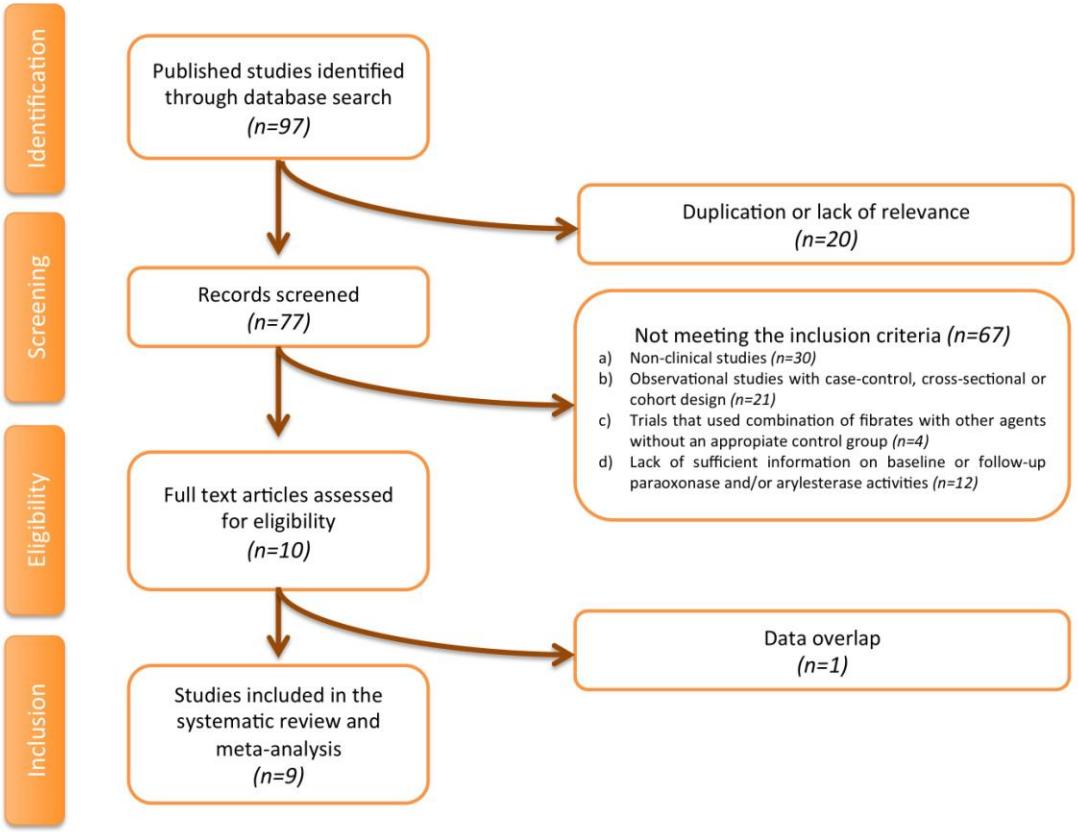
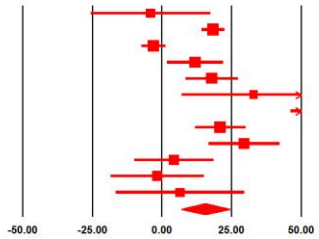


Figure 1

A

Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Agouridis, 2011 [45]	-4.000	10.927	119.401	-25.417	17.417	-0.366	0.714
Balogh, 2001 [51]	18.500	2.041	4.164	14.501	22.499	9.066	0.000
Dullaart, 2009 [46]	-3.000	2.149	4.617	-7.212	1.212	-1.396	0.163
Durrington, 1998a [35]	12.000	5.107	26.078	1.991	22.009	2.350	0.019
Durrington, 1998b [35]	18.000	4.754	22.599	8.683	27.317	3.786	0.000
Paragh, 2000 [52]	33.000	13.110	171.878	7.304	58.696	2.517	0.012
Paragh, 2003 [50]	71.000	12.600	158.760	46.304	95.696	5.635	0.000
Paragh, 2006 [48]	21.000	4.571	20.890	12.042	29.958	4.595	0.000
Phuntuwate, 2008 [47]	29.600	6.500	42.256	16.859	42.341	4.554	0.000
Tsimihodimos, 2003a [49]	4.400	7.269	52.839	-9.847	18.647	0.605	0.545
Tsimihodimos, 2003b [49]	-1.600	8.541	72.944	-18.340	15.140	-0.187	0.851
Tsimihodimos, 2003c [49]	6.600	11.710	137.131	-16.352	29.552	0.564	0.573
Overall	15.643	4.438	19.699	6.944	24.342	3.524	0.000



B

	Point	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Agouridis, 2011 [45]	17.041	4.612	21.266	8.003	26.079	3.695	0.000
Balogh, 2001 [51]	15.557	5.208	27.121	5.350	25.764	2.987	0.003
Dullaart, 2009 [46]	17.568	3.642	13.263	10.430	24.706	4.824	0.000
Durrington, 1998a [35]	16.124	4.869	23.703	6.582	25.667	3.312	0.001
Durrington, 1998b [35]	15.481	4.854	23.563	5.967	24.995	3.189	0.001
Paragh, 2000 [52]	14.608	4.558	20.772	5.675	23.541	3.205	0.001
Paragh, 2003 [50]	12.235	4.125	17.013	4.151	20.320	2.966	0.003
Paragh, 2006 [48]	15.133	4.813	23.164	5.700	24.566	3.144	0.002
Phuntuwate, 2008 [47]	14.257	4.602	21.181	5.237	23.277	3.098	0.002
Tsimihodimos, 2003a [49]	16.730	4.730	22.372	7.460	26.001	3.537	0.000
Tsimihodimos, 2003b [49]	17.123	4.660	21.712	7.991	26.256	3.675	0.000
Tsimihodimos, 2003c [49]	16.271	4.634	21.478	7.188	25.355	3.511	0.000
Overall	15.643	4.438	19.699	6.944	24.342	3.524	0.000

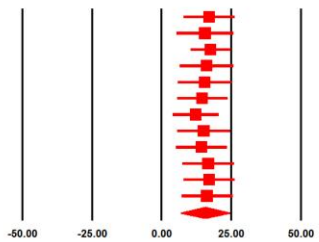
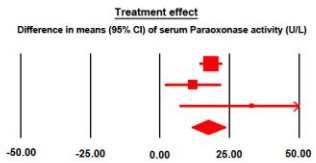


Figure 2

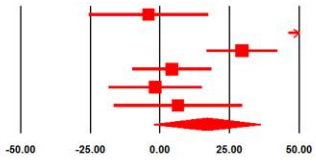
GEMFIBROZIL

Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Balogh, 2001 [51]	18.500	2.041	4.164	14.501	22.499	9.066	0.000
Durrington, 1998a [35]	12.000	5.107	26.078	1.991	22.009	2.350	0.019
Paragh, 2000 [52]	33.000	13.110	171.878	7.304	58.696	2.517	0.012
Overall	17.534	3.088	9.533	11.483	23.586	5.679	0.000



FENOFIBRATE

	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Agouridis, 2011 [45]	-4.000	10.927	119.401	-25.417	17.417	-0.366	0.714
Paragh, 2003 [50]	71.000	12.600	158.760	46.304	95.696	5.635	0.000
Phuntuwate, 2008 [47]	29.600	6.500	42.256	16.859	42.341	4.554	0.000
Tsimihodimos, 2003a [49]	4.400	7.269	52.839	-9.847	18.647	0.605	0.545
Tsimihodimos, 2003b [49]	-1.600	8.541	72.944	-18.340	15.140	-0.187	0.851
Tsimihodimos, 2003c [49]	6.600	11.710	137.131	-16.352	29.552	0.564	0.573
Overall	16.941	9.700	94.099	-2.071	35.954	1.746	0.081



BEZAFIBRATE

	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Dullaart, 2009 [46]	-3.000	2.149	4.617	-7.212	1.212	-1.396	0.163
Durrington, 1998b [35]	18.000	4.754	22.599	8.683	27.317	3.786	0.000
Overall	7.072	10.491	110.067	-13.491	27.634	0.674	0.500

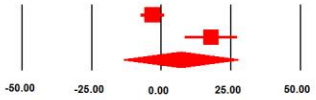


Figure 3

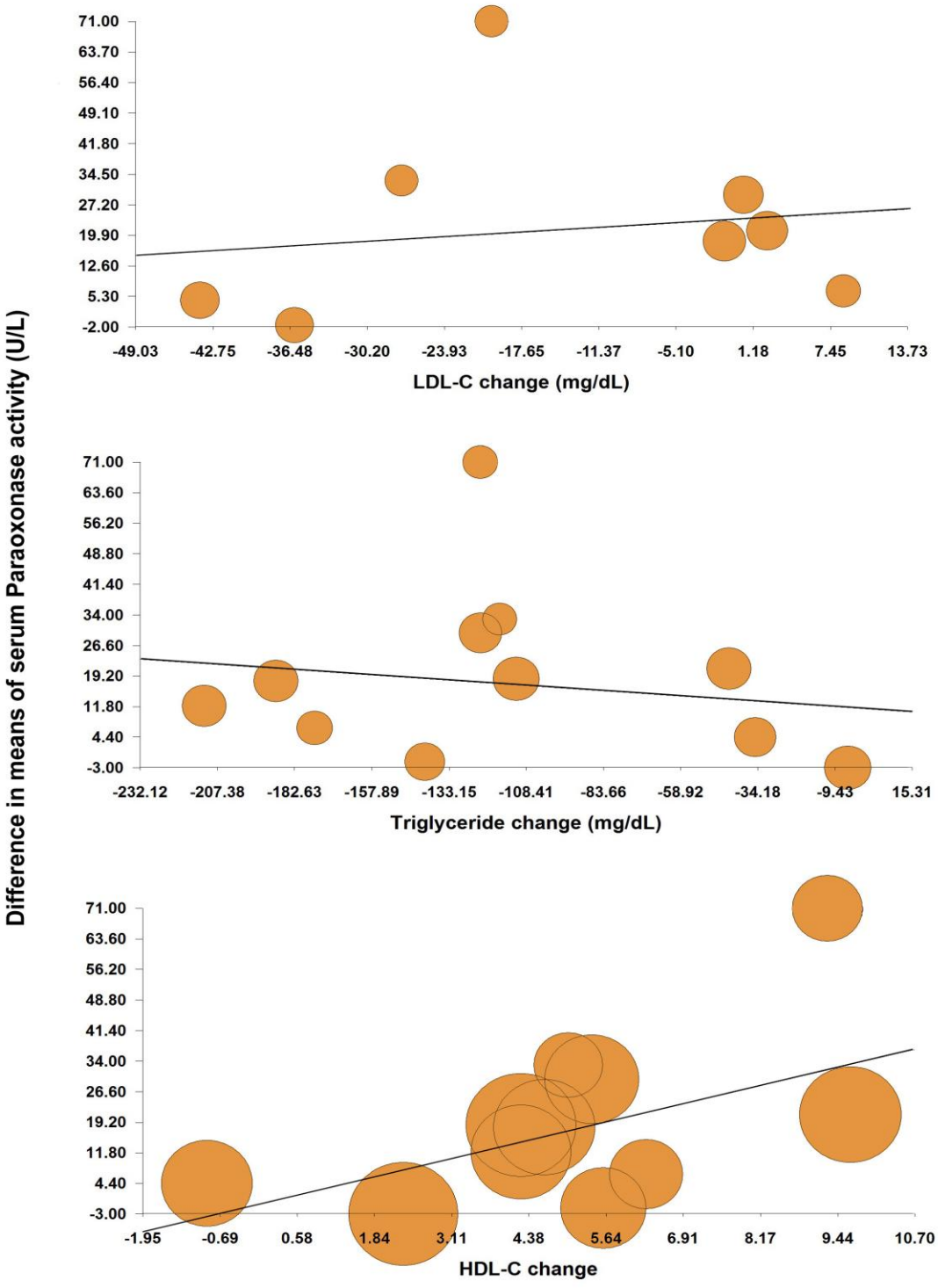


Figure 4

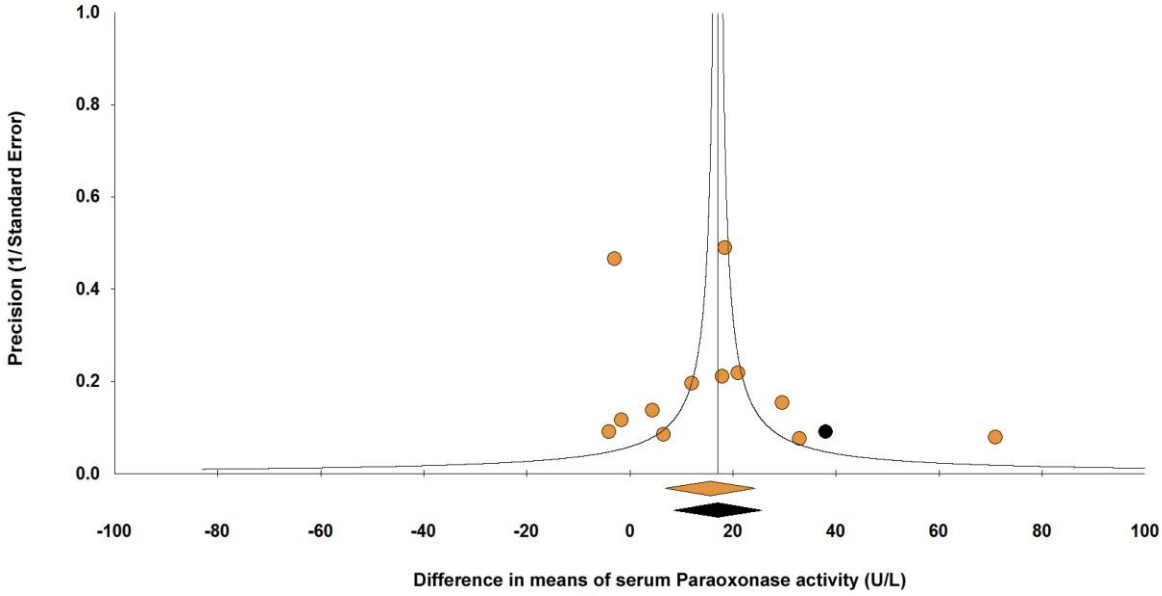


Figure 5

Table1. Demographic characteristics in the eligible studies.

First author	Agouridis [45]	Dullaart [46]	Phuntuwate [47]	Paragh [48]	Tsimihodimos [49]	Paragh [50]	Balogh [51]	Paragh [52]	Durrington [35]
Year	2011	2009	2008	2006	2003	2003	2001	2000	1998
Location	Greece	Netherlands	Thailand	Hungary	France	Hungary	Hungary	Hungary	United Kingdom
Study design	Randomized, open-label, parallel-group trial	Randomized, placebo-controlled, crossover trial	Open-label, non-comparative and intent to treat trial	Open-label, prospective, self-control trial	Controlled, parallel-group trial	Open-label, non-comparative trial	Open-label, case-control trial	Non-comparative trial	Double-blind, placebo-controlled crossover trial
Inclusion criteria	LDL-C >160 mg/dl TG > 200 mg/dl	DM2	DLP TC <299 mg/dl TG < 394 mg/dl HDL-C <39 mg/dl	BMI >27, AHT, DLP, Glucose intolerance.	HLD	Type IIB HLD with CHD.	DM2 TG >210mg/dl TC <270 mg/dl Prescribed diet	TG > 210 mg/dl TC > 208.28 mg/dl	LDL-C >174 mg/dl TG >218 mg/dl despite at least 2 months of dietary therapy.
Follow up (months)	3	2	3	3	3	3	3	3	2
Total sample size	90	63	61	51	162	52	56	57	29
Age (years)*	55 ± 11	58 (42-59)	44.0 ± 6.4	51.9 ± 8.1	51 ± 11	52.8 ± 10.8	55.6 ± 9	61 ± 12	56
Gender (% of male)	45 (50%)	63 (100%)	61 (100%)	NS	35 (49.3%)	16 (30.8%)	40 (40%)	26 (45.6%)	22 (75.9%)
Mean BMI* (kg/m^2)	29.33	26.15	26.3	40.13	27.03	27.13	28.38	26.17	28.4
Drug analyzed	Rosuvastatin fenofibrate	Simvastatin bezafibrate	Fenofibrate	Ciprofibrate	Fenofibrate	Fenofibrate	Gemfibrozil	Gemfibrozil	Bezafibrate gemfibrozil
PAC	√	√	√	√	√	√	√	√	√
AAC	√	√		√	√	√			
PCON			√	√		√			

Reference Number	39	40	41	42	43	44	45	46	47

* Age and BMI were measured at the baseline. *Abbreviations*: AAC, arylesterase activity analyzed in the referred study; AHT, arterial hypertension; BMI, body mass index; CHD, coronary heart disease; DLP, dyslipidemia; DM2, diabetes mellitus type 2; HDL-C, high-density lipoprotein cholesterol; HLD, hyperlipidemia; LDL-C, low-density lipoprotein cholesterol; NS, not stated; PAC, paraoxonase activity analyzed in the referred study; PCON, paraoxonase-1 concentration analyzed in the referred study; TC, total cholesterol; TG, triglycerides; Type IIB, combined hyperlipidemia.

Table 2. Characteristics according to the type and duration of treatment.

First author	Agouridis	Dullaart*	Phuntuwate	Paragh	Tsimihodimos	Paragh	Balogh	Paragh	Durrington*
Year	2011	2009	2008	2006	2003	2003	2001	2000	1998
Drug analyzed	Rosuvastatin Fenofibrate	Simvastatin Bezafibrate	Fenofibrate	Ciprofibrate	Fenofibrate	Fenofibrate	Gemfibrozil	Gemfibrozil	Bezafibrate Gemfibrozil
Duration (months)	3	2 + 2	3	3	3	3	3	3	2 + 2 + 2
Groups of study	R (n=30) RF (n=30) RΩ (n = 30)	DM2 (n= 14) Control (n= 49)	n=61	n= 51	IIA Dyslipemia (n=18) IIB Dyslipemia (n=23) IV Dyslipemia (n=30)	n= 52	DM2 (n= 56) Control (n= 44)	n=57	n=29
Daily dose by group	R 40 mg RF 10mg + 200mg RΩ 10mg + 2g	Both groups	Benzafibrate 400mg Simvastatin 40 mg + matching placebo	Micro-coated Fenofibrate 160mg	Ciprofibrate 100mg	Micronized Fenofibrate 100mg	Micronized Fenofibrate 200mg	Gemfibrozil 600mg + 600mg	Gemfibrozil 600mg	Benzafibrate 400mg Gemfibrozil 600mg + 600mg Placebo
Reference Number	45	46	47	48	49	50	51	52	35

* Placebo controlled crossover study.

R, rosuvastatin; RF, Rosuvastatin + Fenofibrate; RΩ, Rosuvastatin + Omega3 fatty acids.

Table 3. Quality of bias assessment of the included studies according to the Cochrane guidelines..

Study	Random sequence generation	Allocation concealment	Selective reporting	Other bias	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data
Agouridis et al. (2011)	U	U	L	L	H	H	U
Dullaart et al. (2009)	U	U	L	U	U	U	U
Durrington et al. (1998a)	U	U	L	U	L	U	L
Durrington et al. (1998b)	U	U	L	U	L	U	L

* L, low risk of bias; H, high risk of bias; U, unclear risk of bias.