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Comparative Analysis of the Effects of Fish Oil and Fenofibrate on Plasma Metabolomic Profiles in Overweight and Obese Individuals

*Charlotte C.J.R. Michielsen, Roland W.J. Hangelbroek, Marjolijn C.E. Bragt, Elwin R. Verheij, Suzan Wopereis, Ronald P. Mensink, and Lydia A. Afman**

Scope: The drug fenofibrate and dietary fish oils can effectively lower circulating triglyceride (TG) concentrations. However, a detailed comparative analysis of the effects on the plasma metabolome is missing.
Methods and Results: Twenty overweight and obese subjects participate in a double-blind, cross-over intervention trial and receive in a random order 3.7 g day^{-1} n-3 fatty acids, 200 mg fenofibrate, or placebo treatment for 6 weeks. Four hundred twenty plasma metabolites are measured via gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS). Among the treatments, 237 metabolites are significantly different, of which 22 metabolites change in the same direction by fish oil and fenofibrate, including a decrease in several saturated TG-species. Fenofibrate additionally changes 33 metabolites, including a decrease in total cholesterol, and total lysophosphatidylcholine (LPC), whereas 54 metabolites are changed by fish oil, including an increase in unsaturated TG-, LPC-, phosphatidylcholine-, and cholesterol ester-species. All $q < 0.05$.
Conclusion: Fenofibrate and fish oil reduce several saturated TG-species markedly. These reductions have been associated with a decreased risk for developing cardiovascular disease (CVD). Interestingly, fish oil consumption increases several unsaturated lipid species, which have also been associated with a reduced CVD risk. Altogether, this points towards the power of fish oil to change the plasma lipid metabolome in a potentially beneficial way.

1. Introduction

An elevated level of plasma triglycerides (TG) is increasingly recognized as an important, independent, risk factor for cardiovascular disease,[1–3] and it is one of the features of the metabolic syndrome. Lowering plasma TG may therefore be a therapeutic target to lower cardiovascular disease risk.

A class of drugs that effectively lowers plasma TG are fibrates. Fenofibrate in particular is one of the most commonly used fibrates.[4] It selectively activates the ligand-dependent nuclear transcription factor peroxisome proliferator-activated receptor alpha (PPARα). Activation of PPARα leads to regulation of genes involved in lipid metabolism, including fatty acid oxidation and lipoprotein assembly and transport. As a result, fenofibrate inhibits synthesis and secretion of TG by the liver, and stimulates degradation of TG-rich lipoproteins, thereby not only reducing circulating TG, but also very-low and low-density lipoprotein cholesterol.[5–7] Besides the therapeutic approach, plasma TG can

C. C. Michielsen, R. W. Hangelbroek, L. A. Afman
Nutrition, Metabolism and Genomics Group
Division of Human Nutrition and Health
Wageningen University
Stippeneng 4, Wageningen the Netherlands
E-mail: lydia.afman@wur.nl

M. C. Bragt, R. P. Mensink
NUTRIM School of Nutrition and Translational Research in Metabolism
Department of Nutrition and Movement Sciences
Maastricht University Medical Centre+
P.O. Box 616, Maastricht 6200 MD, the Netherlands

E. R. Verheij, S. Wopereis
Unit Healthy Living
Netherlands Organisation for Applied Scientific Research (TNO)
Zeist 3704 HE, the Netherlands

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also effectively be lowered via dietary strategies in the form of dietary n-3 fatty acids.[8–10] Fish oil specifically contains high amounts of n-3 fatty acids in the form of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Like fenofibrate, these dietary n-3 fatty acids can act as ligands for transcription factor PPARα.[11,12] In addition, they can bind with high affinity to PPARγ and PPARδ, two other isotypes of PPARs that are also involved in lipid metabolism.[13] However, while the TG-lowering effects of fenofibrate have been attributed to PPARα activation, the TG-lowering effects of fish oil likely occur independent of PPARα activation, as significant TG reductions have been observed in PPARα-deficient mice models upon fish oil treatment.[14] Instead, the TG-lowering effects of fish oil may be caused via a reduction of ApoB synthase in the liver, thereby impairing VLDL assembly and secretion,[15–18] resulting in reduced TG levels.

The TG-lowering effects of both fenofibrate and fish oils are well known.[8,19] Indeed, a placebo-controlled cross-over study by Bragt et al.[20] demonstrated that both fenofibrate and fish oil reduced plasma TG concentrations. Additionally, they observed an increase in HDL cholesterol, and a reduction sE-selectin, VLDL particle numbers, and LDL cholesterol by fenofibrate, whereas fish oil increased LDL cholesterol and large HDL particle numbers. However, a more detailed comparative analysis of the effects of fenofibrate and fish oil on plasma metabolomic profiles, including several lipid classes and lipid species was not performed. Therefore, our aim was to gain more comprehensive insights into biochemical changes underlying the observed effects of fenofibrate and fish oil on cardiovascular risk factors, and to explore which changes are shared between fenofibrate and fish oil. For this, we performed metabolic profiling using gas chromatography - mass spectrometry (GC-MS) as well as liquid chromatography (LC)-MS platforms, in plasma samples from the study of Bragt and colleagues.[20]

2. Results

Ten men and ten women completed the trial. Compliance of the subjects was good, as calculated mean daily intakes of capsules was 95% or higher during all three intervention periods. The study participants were on average 52 years old, and had a BMI of 33 ± 5 kg m^{-2}, and a baseline TG concentration of 1.63 ± 0.59 mmol L^{-1}. Other baseline characteristics of the study participants, and the effects on the main outcome parameters are reported elsewhere.[20]

2.1. Effects on Metabolic Profiles

Two subjects had missing plasma samples after the fenofibrate intervention, and one subject had missing plasma samples after the placebo intervention. Their remaining samples were included in the univariate analysis. In total 442 metabolites, including 22 calculated ratios, were measured using the FFA (42), GC-MS (204) and LC-MS (196) platforms (Table S1, Supporting Information). Of these, the precise identity of 41 metabolites was not established (all GCMS data). sPLS-DA analysis led to a best fitting model consisting of 1 component, a kappa of 0.5, an eta of 0.9, and an area under the ROC curve of 1, indicating a perfect ability to place the right subject in the right treatment group. Due to the high regularization parameter *eta*, only nine metabolites were needed to consistently separate the fenofibrate and fish oil intervention responses, namely C20:5ω3/C20:3ω6 ratio, C20:4ω6/C20:5ω3 ratio, C20:5ω3/C20:4ω3 ratio, C22:6ω3, "unknown_59b," phosphatidylcholine (PC) (36:5), PC(38:6), PC(40:6), and TG(40:6).

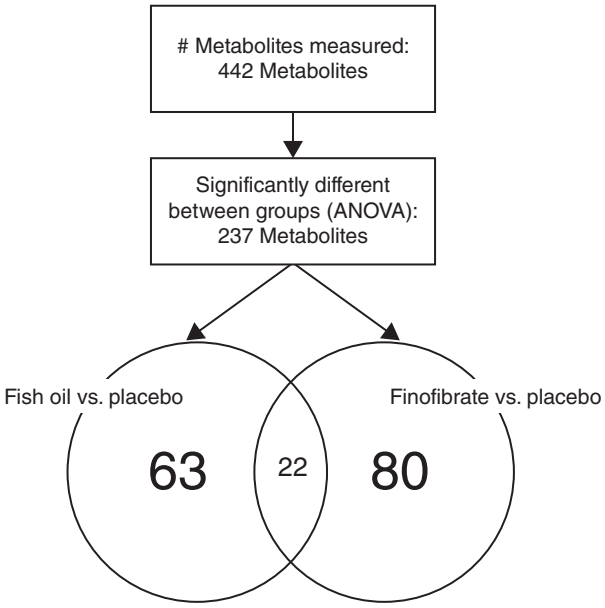

Figure 1. Flow chart of the number of metabolites differentially changed between the interventions. Main effects at the end of each intervention period were tested with an ANOVA. Linear mixed models were used to assess between intervention effects, using treatment as fixed factor and subject number as random factor. Significant metabolites were selected using false discovery rate adjusted *F*-statistic (*q*-value <0.05).

Subsequent univariate analysis on the 442 metabolites and calculated ratios showed that after adjustment for multiple testing, 237 metabolites were significantly different among the treatments (ANOVA, *q*-value <0.05, **Figure 1**). Compared to placebo, 85 metabolites were significantly different after the fish oil treatment, and 102 metabolites were significantly different after the fenofibrate treatment.

2.2. Effect on Lipid Species According to their Degree of Unsaturation

In **Figure 2**, the lipid fraction of the metabolites that were significantly different among the treatments (ANOVA, *q*-value <0.05) are presented, ordered according to their number of double bonds. This figure shows that several saturated lipid species were decreased by fenofibrate only. Additionally, the figure illustrates that both fenofibrate and fish oil treatments significantly decreased 30 lipid species containing four or fewer double bonds. For 14 out of these 30 (e.g., total TG and eight TG-species), the effects were equal for both treatments, and for 13 out of 30, the decrease induced by the fish oil treatment was significantly larger than the effects of the fenofibrate treatment. The fish oil treatment additionally induced a significant increase

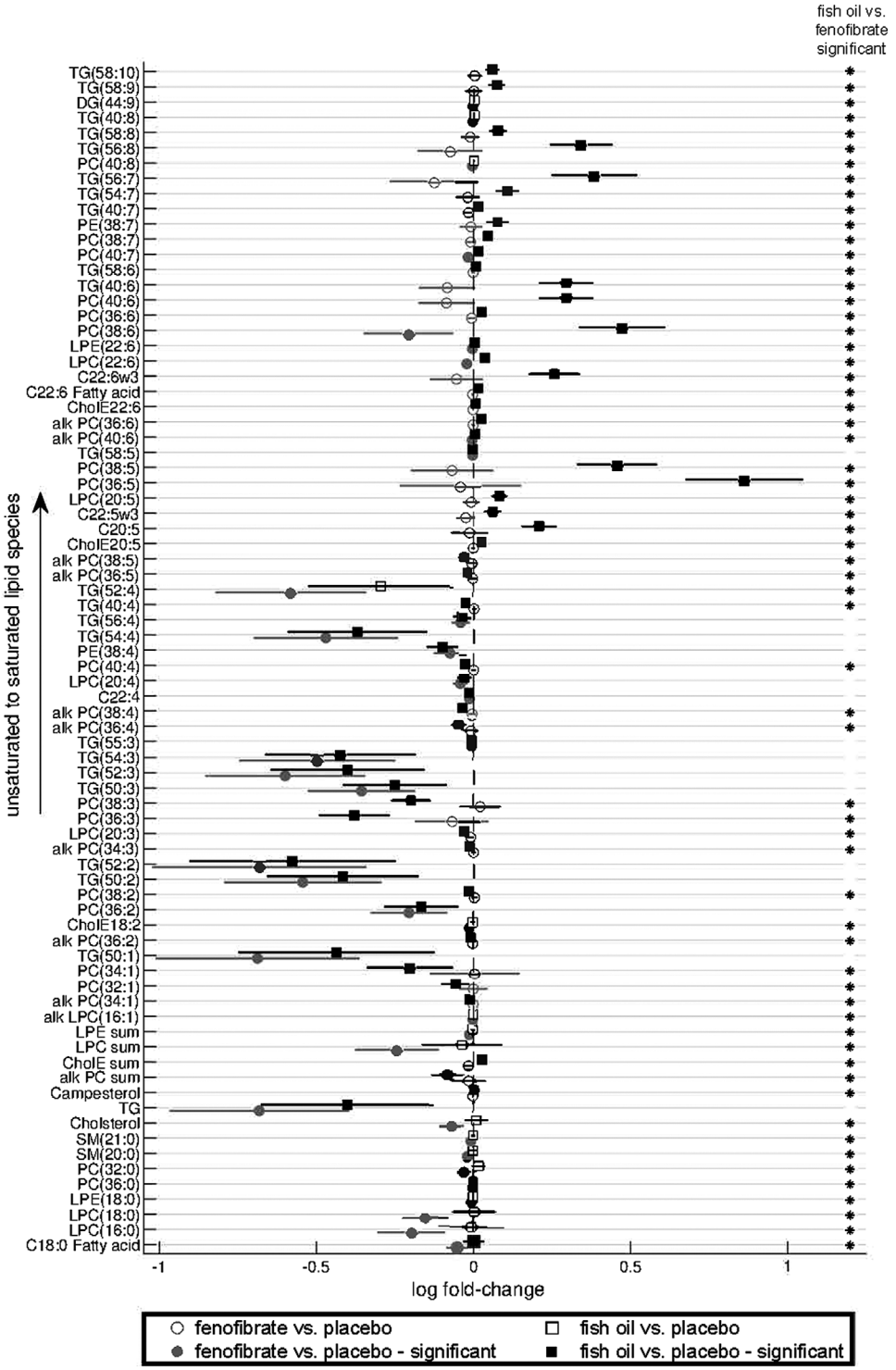

Figure 2. Forrest plot of the effects of the interventions on lipid species. Lipid species that were significantly different among the treatments (ANOVA, q-value <0.05) are presented ordered according to degree of unsaturation. Linear mixed models were used to assess between intervention effects. Significant indicates a q-value < 0.05.

Table 1. Effects of the fenofibrate and fish oil interventions on non-lipid metabolites and lipid metabolite ratios that were significantly different among the treatments (ANOVA, *q*-value <0.05).

		Fenofibrate vs placebo	Fish oil vs placebo	Fish oil vs Fenofibrate
Lipid ratios	campesterol /cholesterol ratio		*	*
	LPC(16:1)/LPC(16:0)	*	*	*
	LPC(18:1)/LPC(18:0) ratio		*	*
	LPC(36:4)/LPC(16:0) ratio		*	*
	SM(d16:1/20:0)	*		*
	C18:2/C20:3w6 ratio	*		*
	C20:3w9/C20:4w6 ratio		*	*
	C20:4w6/C20:3w6 ratio	*		*
	C20:4w6/C20:5 ratio		*	*
	C20:5/C20:3w6 ratio		*	*
	C20:5/C20:4w3 ratio		*	*
	C22:5w6/C22:6w3 ratio		*	*
Other metabolites	1,5-Anhydro-D-Glucitol	*		*
	2,3,4-Trihydroxybutanoic acid	*		*
	2,3-Dihydroxybutanoic acid	*		*
	2,4-Dihydroxybutanoic acid	*		*
	32006/01.07.02 uk x 21		*	*
	32006\01.08.02 uk x 55		*	*
	4-oxo-proline	*		*
	ascorbic acid	*		*
	methyl uric acid, isomeer 1	*	#	*
	methyl uric acid, isomeer 2	*		*
	P7478_uk 05	*		*
	P7502_uk 05	*		*
	P7881_uk 22	*		*
	Tryptophan/other amino acids	*		*
	unknown 59b		*	*
	Uric acid	*		*
	Uridine	*		*

Linear mixed models were used to assess between intervention effects. Significant indicates a *q*-value < 0.05; * *q*-value <0.05; # 0.05 ≤ *q*-value < 0.06;

Mean Log Fold Change

-0.1 0 0.1

in lipid species containing five double bonds or more, while the fenofibrate treatment had no effects or induced a significant decrease. Fish oil treatment also slightly, but significantly, increased the sum of cholesterol esters, and the fenofibrate treatment significantly decreased total cholesterol.

2.3. Other Metabolites Modulated by Fish Oil and Fenofibrate

In **Table 1**, the lipid ratio's and non-lipid species that were significantly different among the treatments (ANOVA, *q*-value <0.05) are presented. Fenofibrate decreased uric acid and its derivative methyluric acid, as well as ascorbic acid, the ratio of tryptophan to other amino acids, 1,5-anhydro-D-glucitol, and 2,3,4-trihydroxybutanoic acid, and increased 2,4-dihydroxybutanoic acid, and 2,3-dihydroxybutanoic acid. The metabolite "unknown 59b" was increased by fish oil only. Based on the molecular weight this metabolite is possibly related to vitamin E, which might represent the tocopherol content of the fish oil capsules. Individual changes in all metabolites that were significantly different among the treatments, thus including the lipid fraction, are presented in Tables S2–S5, Supporting Information. The variation in changes among the participants was low, indicating that both the fenofibrate and fish oil treatments induced robust effects.

Table 1 Effects of the fenofibrate and fish oil interventions on non-lipid metabolites and lipid metabolite ratios that were significantly different among the treatments (ANOVA, *q*-value <0.05)

3. Discussion

In this study, we characterized and compared the effects of the PPARα agonist fenofibrate (200 mg day^{-1}) with a dietary PPARα agonist n-3 LCPUFAs (3.7 g day^{-1}) on plasma metabolic profiles

in overweight and obese subjects. We demonstrated that both fish oil and fenofibrate altered the metabolic plasma profile markedly, with clear intervention-specific and shared effects between the two treatments.

Fenofibrate and the fish oil intervention both reduced total TG, and several TG-species containing less than five double bonds. The total TG-lowering effects of fenofibrate as well as fish oil are well documented[8,19] and are in line with what was previously reported in the same study population.[20] The effects of fenofibrate have previously been ascribed to an increased TG clearance by activation of lipoprotein lipase via PPAR-mediated decrease of *APOC3* gene expression,[21–24] and a decreased TG production and secretion from the liver via upregulation of fatty acid beta oxidation via PPAR activation.[25] The TG-lowering effects of fish oil, on the other hand, have previously, at least partly, been ascribed to a reduction of ApoB synthase in the liver, thereby impairing VLDL assembly and secretion.[15–18] We here demonstrate that besides a decrease in total TG, both fenofibrate and fish oil reduced specifically the TG-species containing fewer than 5 double bonds, indicating a remodeling of the TG species towards a lower saturation status of the TG fraction. As higher proportions of saturated fatty acids have been positively associated with obesity and insulin resistance,[26–29] the observed shift towards a lower saturated state points towards a more beneficial profile. In addition, TG-species with a high saturated state have shown consistent and strong positive associations with cardiovascular disease risk.[30] The observed decrease in saturated TG-species might have been caused by an increased activity of enzymes responsible for desaturation of fatty acids, such as fatty acid desaturase 1 (FADS1) and FADS2, which are known targets of the transcriptional regulator PPAR.[31] In line with this, the fenofibrate intervention decreased the C18:2ω6/C20:3ω6 ratio, which indicates an estimated increase in the activity of the enzyme delta 6 desaturase (D6D), encoded by the FADS2 gene. The fish oil intervention did not affect this ratio, but did increase the C20:5ω3/C20:4ω3 ratio, which indicates an estimated increase in the activity of delta 5 desaturase (D5D). This enzyme, encoded by the FADS1 gene, is required for the synthesis of highly unsaturated fatty acids.[32] An increase in D5D activity, as estimated by this ratio, has been described upon replacing saturated fatty acids with PUFA.[33] Fenofibrate, on the other hand, decreased the activity of D5D, as the fenofibrate intervention decreased the C20:4ω6/C20:3ω6 ratio, another ratio used for estimating D5D activity. Thus, the activity of D5D was estimated to be increased after the fish oil intervention and decreased after the fenofibrate intervention. Therefore, the question remains whether this effect of fish oil on D5D activity can indeed be attributed to PPAR activation. Especially since fish oil is not only a ligand for PPARα, but can also activate other metabolic pathways involved in lipid metabolism.[34] For example, fish oils have shown to inhibit the sterol regulatory element-binding protein 1c (SREBP-1c),[35] and can thereby inhibit de novo lipogenesis in the liver. This might also partially explain the observed decrease in the relatively saturated TG-species.

Thus, both fenofibrate and fish oil reduced the total TG class, and the saturated status of the TG fraction. The effect of fenofibrate is likely explained by PPAR-mediated expression of the FADS2 gene, which is involved in desaturase enzyme activities of fatty acids. The effect of fish oil is possibly partly explained via a PPAR-mediated increase in FADS1 expression, but other mechanistic pathways might have also played a role.

Furthermore, fenofibrate decreased the total sum of LPC-species. LPC-species have been found to be increased in atherosclerotic plaques,[36] and have shown positive associations with obesity and features of subcutaneous obesity.[37] In accordance with previous studies,[38–40] fenofibrate decreased uric acid, as well as its derivative methyluric acid, caused by an increase in renal urate excretion. Lastly, fenofibrate decreased 1,5-anydro-D-glucitol, a marker of glycemic control in diabetes patients,[41] which has not previously been reported to be decreased by fenofibrate.

The fish oil intervention reduced total TG and caused a shift within the TG-class by both decreasing the more saturated TG-species, as well as increasing unsaturated TG-species. Next to lower proportions of the more saturated fatty acids, higher proportions of longer chain ω3 and ω6 polyunsaturated fatty acids in plasma TG have also been inversely associated with obesity and insulin resistance.[26–29,42] This might thus point towards an even more beneficial profile[26,43] compared to the profile induced by the fenofibrate treatment. In line with our results, similar increases in desaturation of TG-species in the circulation have previously been observed in several dietary intervention studies examining the effects of either fish oils or fatty fish intake.[44,45] Of note, while we observed a potentially beneficial shift upon the fish oil intervention by a decrease in more saturated TG-species and an increase in unsaturated lipid species, Bragt et al.[20] reported that the number of very small-, small-, large and total LDL particles, and total LDL cholesterol increased after the fish oil intervention in the same population. The increase in LDL cholesterol after high intakes of fish oil has been observed in several other studies.[46] It remains to be investigated how the observed changes in saturation state of several lipid species relate to the observed increase in LDL cholesterol in terms of health effects.

Apart from the PPAR-mediated activation of FADS1 and the activation of other regulatory pathways (e.g., SREBP-1c), the observed increase by fish oil in unsaturated TG-, PC-, LPC-, and cholesterol ester-species may have been caused by an increased supply of fatty acids containing a high number of double bonds via the fish oil, that can subsequently be incorporated in circulating lipid species. This substantiates the suggestion that the average dietary fatty acid composition in the circulation represents the dietary intake during the last day(s).[33,47] In line with our results, the increase in cholE(20:5ω3) was also observed after 8 weeks of consumption of a healthy diet containing fatty fish in subjects with features of the metabolic syndrome,[48] and after 7 weeks of a lower dose of fish oil (1.6 g day^{-1} EPA+DHA).[49] Similar to our study, the latter study also reported an increase in LPC(22:6ω3).[49] The exact consequences of the changes in unsaturated PC- and LPC-species for cardiovascular health remain to be elucidated.

Thus, apart from the shared effects of fenofibrate and fish oil on total TG and the more saturated TG-species, the fish oil intervention additionally increased several unsaturated TGs, PCs, LPCs, and cholesterol ester-species. This is likely mainly due to an increased incorporation in the circulating lipid species of

the consumed unsaturated fatty acids, EPA and DHA, but may also partly be regulated by PPAR-mediated activation of genes involved in fatty acid desaturase activity. Albeit that several stable isotope tracer studies have shown that the synthesis of EPA and especially DHA is known to be very limited in humans, as reviewed in.[50,51]

Next to EPA and DHA, the fish oil capsules contained a mix of natural tocopherols totaling to an amount of around 21 mg per day (~32 IU), which was potentially reflected in the metabolic profiling by compound "unknown 59b." As the antioxidant vitamin E was not present in the fibrate supplement, effects of vitamin E on plasma metabolites in the fish oil intervention cannot be excluded, although thus far no known effects have been reported. The amount of fish oil (3.7 g) taken daily was very high compared to the adequate intake of 0.25 g as set by the EFSA.[52] This might partly explain why the observed effects of fish oil on total TG and saturated TG-species were robust. For the treatment of patients with hypertriglyceridemia, high doses (2-4 g day^{-1} total EPA+DHA) are recommended by the American Heart Association,[9] and in the landmark REDUCE-IT trial,[53] a dose of 4 g day^{-1} n-3 fatty acids has shown to be effective as secondary prevention of cardiovascular events in high-risk patients treated with statin.

A limitation of the methods used in this study is that we could only identify the sum compositions of the lipid species by the used platforms, and not the precise identity of the molecular lipid species.[54] The strength of this study lies in its design; by including both the fenofibrate and the fish oil treatment in a crossover study, we could directly compare the effects of a pharmacological treatment to the effects of a nutrient intervention. Lastly, by combining two LC-MS platforms and one GC-MS platform, we obtained a comprehensive view of the individual metabolic changes caused by fish oil and fenofibrate in the circulation.

In summary, the current study in which we compared the effects of a 6-week intervention with either fenofibrate or fish oil revealed shared and specific effects of both interventions on plasma metabolome, especially on the lipid metabolites. Both fenofibrate and fish oil reduced the total TG class and the saturated status of the TG fraction. The decrease in the relatively saturated TG-species by fenofibrate can almost entirely be attributed to PPARα activation, while this effect in the fish oil intervention is potentially mediated via the activation of other mechanistic pathways. The fish oil intervention additionally increased several unsaturated TGs, PCs, LPCs, and cholesterol ester-species. This is likely mainly due to an increased incorporation in the circulating lipid species of the consumed unsaturated fatty acids, EPA and DHA, but might also partly be regulated by the combined effects of PPAR-mediated activation of genes involved in fatty acid desaturase activity.

In conclusion, both fenofibrate, a drug, and fish oil, a nutrient, resulted in a similar beneficial decrease in relatively saturated TG-species, that have been associated with a decreased risk for developing CVD. Interestingly, the fish oil consumption additionally induced an increase in the unsaturated lipid fraction, which also have been associated with a reduced CVD risk. Altogether, this points towards the power of fish oil to change the plasma lipid metabolome in a potentially beneficial way.

4. Experimental Section

Subjects: For the current manuscript, the authors used samples collected during a randomized, double-blind, placebo-controlled crossover designed human intervention study, examining the effects of fenofibrate and fish oil on inflammatory parameters, vascular function and lipoprotein profiles in overweight and obese subjects. This study was performed in 2007 at Maastricht University and was approved by the Medical Ethics Committee of Maastricht University and was registered at EudraCT 2006-005743-28. An extensive description of the study design, recruitment, methods, and results of the primary outcome measures can be found elsewhere.[20] In short, 26 subjects (BMI $\geq$27 kg m^{-2}) were assigned in random order to a fish oil, a fenofibrate, and a placebo intervention for 6 weeks with a wash-out period of at least 2 weeks in between the intervention periods. During the fish oil intervention, subjects consumed eight fish oil capsules daily (Marinol C-38, Lipid Nutrition, Wormerveer, the Netherlands), providing approximately 3.7 g day^{-1} n-3 long chain polyunsaturated fatty acids (LCPUFA; 1.7 g day^{-1} EPA and 1.2 g day^{-1} DHA,) and two capsules placebo-matching fenofibrate (200 mg day^{-1} cellulose). During the fenofibrate period, subjects consumed two capsules providing 200 mg day^{-1} micronized fenofibrate (Lipanthyl, Fournier Laboratories, Dijon, France) and eight placebo-matching fish oil capsules (containing 80% High Oleic Sunflower Oil, HOSO). During the placebo period, subjects received eight HOSO capsules and two cellulose capsules. The doses of 200 mg day^{-1} and 3.7 g day^{-1} for the fenofibrate and the fish oil interventions, respectively, were chosen, because these doses have shown to effectively lower TG. Moreover, Bragt et al.[20] showed that with these doses a similar decrease in TG concentration was found. The power calculation was based on the primary outcome of the study, which was to detect a true difference of 0.20 mmol L^{-1} in TG concentrations between the treatments with a power of 80%.

Blood Sampling: In weeks 5 and 6 of each intervention period fasting EDTA, heparin, and NaF blood samples were taken in BD vacutainer tubes (Becton Dickinson Company, NJ). Plasma aliquots were snap-frozen and stored at -80°C until further analyses.

Plasma Metabolic Profiling: Plasma samples from weeks 5 and 6 were pooled and changes in the composition of plasma free fatty acids (TNO, Zeist, the Netherlands) were analyzed using liquid chromatography mass spectrometry (LC-MS) as described by Wopereis and colleagues.[55] Changes in polar plasma lipids were analyzed using another LC-MS platform (University of Leiden, The Netherlands), and further changes in metabolite profiles were analyzed using gas chromatography mass spectrometry (GC-MS) (TNO, Zeist, the Netherlands), both as described in,[55] where also detailed information on analytical performance of the metabolic platforms can be found. In short, the performance was assessed by frequent use of Quality Control (QC) samples, and metabolites with very high imprecision (e.g., a Relative Standard Deviation higher than 50%), were removed from the data, unless large differences between the intervention groups were found. Together, the three platforms covered a total of 442 plasma metabolites, including 22 calculated ratios. Of these metabolites, 401 could be identified, including a wide variety of chemical classes (Table S1, Supporting Information).

Metabolic Profiling Analysis: For the analysis of the metabolomics data, log transformed data was used. Main effects at the end of the three intervention (i.e., fenofibrate, fish oil, and placebo) periods were tested with an ANOVA. Subsequently, linear mixed models were used to assess between intervention effects, with treatment as fixed effect and subject number as random effect. Hereby, differences between pairs of the interventions were checked (i.e., fenofibrate vs placebo, fish oil vs placebo, and fish oil vs fenofibrate). Significant metabolites were selected using the false discovery rate (FDR) adjusted *F*-statistic[56] *q*-value <0.05. Linear mixed model analysis was performed using the *lme4*[57] and *emmeans*[58] R libraries. Furthermore, it was examined whether observed changes in metabolic profiles for fish oil and fenofibrate relative to the placebo group could be separated by using sparse partial least squares discriminant analysis (sPLS-DA). The sPLS-DA model was made using the *caret*[59] and *spls*[60] R library. Optimal hyperparameters were evaluated using grid search and selected

based on the highest area under the receiver operating characteristic curve (AUROC) during five times repeated five-fold cross-validation. All analyses were done using R v4.0.2.[61]

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Authors Contributions

M.C.E.B and R.P.M. designed and executed the study. E.R.V. and S.W. performed the plasma metabolic profiling using the FFA LCMS and the GCMS platforms. S.W. cleaned the metabolomics dataset and calculated the metabolite ratios. C.C.J.R.M. and R.W.J.H. analyzed the data. C.C.J.R.M. wrote the manuscript, which was critically reviewed and improved by S.W., R.P.M., and L.A.A. All authors read and approved the final manuscript. The study and the metabolomic analyses were funded by the Nutrigenomics Consortium (NGC) of Top Institute Food and Nutrition (TIFN). This project is part of the FoodBAll project. FoodBAll is a project funded by the BioNH call (grant number 529051002) under the Joint Programming Initiative, "A Healthy Diet for a Healthy Life."

Data Availability Statement

Data available on request from the authors.

Keywords

clinical trial, human, lipidomics, nutrigenomics, peroxisome proliferator-activated receptor α

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[1] M. Miller, N. J. Stone, C. Ballantyne, V. Bittner, M. H. Criqui, H. N. Ginsberg, A. C. Goldberg, W. J. Howard, M. S. Jacobson, P. M. Kris-Etherton, T. A. Lennie, M. Levi, T. Mazzone, S. Pennathur, *Circulation* **2011**, *123*, 2292.
[2] A. Boullart, J. De Graaf, A. Stalenhoef, *Biochim. Biophys. Acta (BBA)* **2012**, *1821*, 867.
[3] M. Budoff, *Am. J. Cardiol.* **2016**, *118*, 138.
[4] T. Filippatos, H. J. Milionis, *Expert Opin. Invest. Drugs* **2008**, *17*, 1599.
[5] K. Tziomalos, V. G. Athyros, *Int. J. Nanomed.* **2006**, *1*, 129.
[6] R. S. Birjmohun, B. A. Hutten, J. J. Kastelein, E. S. Stroes, *J. Am. Coll. Cardiol.* **2005**, *45*, 185.
[7] B. Staels, J. Dallongeville, J. Auwerx, K. Schoonjans, E. Leitersdorf, J.-C. Fruchart, *Circulation* **1998**, *98*, 2088.
[8] W. S. Harris, *Am. J. Clin. Nutr.* **1997**, *65*, 1645S.
[9] A. C. Skulas-Ray, P. W. F. Wilson, W. S. Harris, E. A. Brinton, P. M. Kris-Etherton, C. K. Richter, T. A. Jacobson, M. B. Engler, M. Miller, J. G. Robinson, C. B. Blum, D. Rodriguez-Leyva, S. D.d. Ferranti, F. K. Welty, *Circulation* **2019**, *140*, e673.
[10] M. Arca, C. Borghi, R. Pontremoli, G. M. De Ferrari, F. Colivicchi, G. Desideri, P. L. Temporelli, *Nutr. Metab. Cardiovasc. Dis.* **2018**, *28*, 197.
[11] U. Kintscher, R. E. Law, *Am. J. Physiol. Endocrinol. Metab.* **2005**, *288*, E287.
[12] L. M. Sanderson, P. J. de Groot, G. J. Hooiveld, A. Koppen, E. Kalkhoven, M. Müller, S. Kersten, *PLoS One* **2008**, *3*, e1681.
[13] S. Kersten, B. Desvergne, W. Wahli, *Nature* **2000**, *405*, 421.
[14] J. Dallongeville, E. Baugé, A. Tailleux, J. M. Peters, F. J. Gonzalez, J.-C. Fruchart, B. Staels, *J. Biol. Chem.* **2001**, *276*, 4634.
[15] C. Lang, R. Davis, *J. Lipid Res.* **1990**, *31*, 2079.
[16] D. C. Chan, G. F. Watts, P. H. R. Barrett, L. J. Beilin, T. G. Redgrave, T. A. Mori, *Diabetes* **2002**, *51*, 2377.
[17] P. Nestel, W. Connor, M. Reardon, S. Connor, S. Wong, R. Boston, *J. Clin. Invest.* **1984**, *74*, 82.
[18] D. C. Chan, G. F. Watts, T. A. Mori, P. H. R. Barrett, T. G. Redgrave, L. J. Beilin, *Am. J. Clin. Nutr.* **2003**, *77*, 300.
[19] R. S. Rosenson, D. A. Wolff, A. L. Huskin, I. B. Helenowski, A. W. Rademaker, *Diabetes Care* **2007**, *30*, 1945.
[20] M. C. E. Bragt, R. P. Mensink, *Nutrition* **2012**, *22*, 966.
[21] Y. Park, P. G. Jones, W. S. Harris, *Am. J. Clin. Nutr.* **2004**, *80*, 45.
[22] S. Khan, A. M. Minihane, P. J. Talmud, J. W. Wright, M. C. Murphy, C. M. Williams, B. A. Griffin, *J. Lipid Res.* **2002**, *43*, 979.
[23] H. E. Bays, A. P. Tighe, R. Sadovsky, M. H. Davidson, *Expert Rev. Cardiovasc. Ther.* **2008**, *6*, 391.
[24] J.-C. Fruchart, B. Staels, P. Duriez, *Pharmacol.l Res.* **2001**, *44*, 345.
[25] M. H. Davidson, *Am. J. Cardiol.* **2006**, *98*, 27.
[26] B. Vessby, *Curr. Opin. Lipidol.* **2003**, *14*, 15.
[27] L. H. Storlien, L. A. Baur, A. D. Kriketos, D. A. Pan, G. J. Cooney, A. B. Jenkins, G. D. Calvert, L. V. Campbell, *Diabetologia.* **1996**, *39*, 621.
[28] E. Warensjö, U. Risérus, B. Vessby, *Diabetologia.* **2005**, *48*, 1999.
[29] J. Graessler, D. Schwudke, P. E. H. Schwarz, R. Herzog, A. Shevchenko, S. R. Bornstein, *PLoS One* **2009**, *4*, e6261.
[30] C. Stegemann, R. Pechlaner, P. Willeit, S. R. Langley, M. Mangino, U. Mayr, C. Menni, A. Moayyeri, P. Santer, G. Rungger, T. D. Spector, J. Willeit, S. Kiechl, M. Mayr, *Circulation* **2014**, *129*, 1821.
[31] M. Rakhshandehroo, B. Knoch, M. Müller, S. Kersten, *PPAR Res.* **2010**, *2010*, 612089.
[32] M. T. Nakamura, T. Y. Nara, *Annu. Rev. Nutr.* **2004**, *24*, 345.
[33] B. Vessby, I. B. Gustafsson, S. Tengblad, M. Boberg, A. Andersson, *Ann. N. Y. Acad. Sci.* **2002**, *967*, 183.
[34] Y. Lu, M. V. Boekschoten, S. Wopereis, M. Müller, S. Kersten, *Physiol. Genomics* **2011**, *43*, 1307.
[35] F. W. B. Sanders, J. L. Griffin, *Biol Rev Camb Philos Soc* **2016**, *91*, 452.
[36] C. Stegemann, I. Drozdov, J. Shalhoub, J. Humphries, C. Ladroue, A. Didangelos, M. Baumert, M. Allen, A. H. Davies, C. Monaco, A. Smith, Q. Xu, M. Mayr, *Circ. Cardiovasc. Genet.* **2011**, *4*, 232.
[37] K. H. Pietiläinen, M. Sysi-Aho, A. Rissanen, T. Seppänen-Laakso, H. Yki-Järvinen, J. Kaprio, M. Orešič, *PLoS One* **2007**, *2*, e218.
[38] M. Elisaf, *Curr. Med. Res. Opinion* **2002**, *18*, 269.
[39] A. Achimastos, E. Liberopoulos, S. Nikas, E. Bairaktari, G. Miltiadous, V. Tsimihodimos, M. Elisaf, *Curr. Med. Res. Opinion* **2002**, *18*, 59.
[40] M. D. Bastow, P. N. Durrington, M. Ishola, *Metab. Clin. Exp.* **1988**, *37*, 217.
[41] T. Yamanouchi, S. Minoda, M. Yabuuchi, Y. Akanuma, H. Akanuma, H. Miyashita, I. Akaoka, *Diabetes* **1989**, *38*, 723.

[42] U. Schwab, T. Seppänen-Laakso, L. Yetukuri, J. Ågren, M. Kolehmainen, D. E. Laaksonen, A.-L. Ruskeepää, H. Gylling, M. Uusitupa, M. Orešič, *PLoS One* **2008**, *3*, e2630.
[43] E. P. Rhee, S. Cheng, M. G. Larson, G. A. Walford, G. D. Lewis, E. McCabe, E. Yang, L. Farrell, C. S. Fox, C. J. O'Donnell, S. A. Carr, R. S. Vasan, J. C. Florez, C. B. Clish, T. J. Wang, R. E. Gerszten, *J. Clin. Invest.* **2011**, *121*, 1402.
[44] G. McCombie, L. M. Browning, C. M. Titman, M. Song, J. Shockcor, S. A. Jebb, J. L. Griffin, *Metabolomics* **2009**, *5*, 363.
[45] M. Lankinen, U. Schwab, M. Kolehmainen, J. Paananen, K. Poutanen, H. Mykkänen, T. Seppänen-Laakso, H. Gylling, M. Uusitupa, M. Orešič, *PLoS One* **2011**, *6*, e22646.
[46] E. M. Balk, G. P. Adams, V. Langberg, C. Halladay, M. Chung, L. Lin, S. Robertson, A. Yip, D. Steele, B. T. Smith, *Evid. Rep. Technol. Assess.* **2016**, *223*, 1.
[47] M. Katan, J. Deslypere, A. Van Birgelen, M. Penders, M. Zegwaard, *J. Lipid Res.* **1997**, *38*, 2012.
[48] M. Lankinen, U. Schwab, A. Erkkilä, T. Seppänen-Laakso, M.-L. Hannila, H. Mussalo, S. Lehto, M. Uusitupa, H. Gylling, M. Orešič, *PLoS One* **2009**, *4*, e5258.
[49] I. Ottestad, S. Hassani, G. I. Borge, A. Kohler, G. Vogt, T. Hyötyläinen, M. Orešič, K. W. Brønner, K. B. Holven, S. M. Ulven, M. C. W. Myhrstad, *PLoS One* **2012**, *7*, e42550.
[50] G. Burdge, *Curr. Opin. Clin. Nutr. Metab. Care.* **2004**, *7*.
[51] E. J. Baker, E. A. Miles, G. C. Burdge, P. Yaqoob, P. C. Calder, *Progr. Lipid Res.* **2016**, *64*, 30.
[52] EFSA Panel on Dietetic Products, Nutrition: Allergies, *EFSA J.* **2010**, *8*, 1461.
[53] D. L. Bhatt, P. G. Steg, M. Miller, E. A. Brinton, T. A. Jacobson, S. B. Ketchum, R. T. Doyle, R. A. Juliano, L. Jiao, C. Granowitz, J.-C. Tardif, C. M. Ballantyne, *N. Engl. J. Med.* **2018**, *380*, 11.
[54] K. Ekroos, M. Jänis, K. Tarasov, R. Hurme, R. Laaksonen, *Curr. Atheroscler. Rep.* **2010**, *12*, 273.
[55] S. Wopereis, C. M. Rubingh, M. J. van Erk, E. R. Verheij, T. van Vliet, N. H. Cnubben, A. K. Smilde, J. van der Greef, B. van Ommen, H. F. Hendriks, *PLoS One* **2009**, *4*, e4525.
[56] Y. Benjamini, Y. Hochberg, *J. Royal Stat. Soc. B (Methodological)* **1995**, *57*, 289.
[57] D. Bates, M. Mächler, B. Bolker, S. Walker, arXiv preprint arXiv:1406.5823 2014, 1.
[58] S. R. Searle, F. M. Speed, G. A. Milliken, *Am. Stat.* **1980**, *34*, 216.
[59] M. Kuhn, *J. Stat. Softw.* **2008**, *28*, 1.
[60] H. Chun, S. Keleş, *J. Royal Stat. Soc. B (Statistical Methodology)* **2010**, *72*, 3.
[61] R Core Team, R: A language and environment for statistical computing **2013**.