Research Article

Short-Term Activation of Peroxisome Proliferator-Activated Receptors α and γ Induces Tissue-Specific Effects on Lipid Metabolism and Fatty Acid Composition in Male Wistar Rats

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Received 17 February 2019; Revised 28 April 2019; Accepted 19 May 2019; Published 12 June 2019

Academic Editor: Stéphane Mandard

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Dietary fatty acids (FAs) affect certain metabolic routes, including pathways controlled by the peroxisome proliferator-activated receptors (PPARs), but tissue-specific effects are not well-defined. Thus, the aim was to compare the metabolic response in hepatic, adipose, and cardiac tissues after treatment with specific PPAR agonists. Male Wistar rats were randomized into three groups: a control group receiving placebo (n=8); a PPARα agonist group receiving WY-14,643 (n=6); and a PPARγ agonist group receiving rosiglitazone (n=6) for 12 days. All animals received a low-fat standard chow diet and were given a daily dose of placebo or agonist orally. Lipids and FA methyl esters were measured in plasma, liver, and heart and gene expression was measured in liver and adipose tissue, while enzyme activities were measured in liver. Treatment with the PPARα agonist was associated with higher liver mass relative to body weight (liver index), lower plasma, and hepatic total cholesterol, as well as lower plasma carnitine and acylcarnitines, compared with control. In heart, PPARα activation led to overall lower levels of free FAs and specific changes in certain FAs, compared with control. Furthermore, β-oxidation in liver and the enzymatic activities of well-known PPARα targeted genes were higher following PPARα administration. Overall, rats treated with the PPARα agonist had higher hepatic saturated FAs (SFAs) and monounsaturated FAs (MUFAs) and lower n-6 and n-3 PUFA, compared with control. Treatment with the PPARγ agonist was associated with a lower liver index, lower plasma triglycerides (TAG) and phospholipids, and higher hepatic phospholipids, compared with control. PPARγ target genes were increased specifically in adipose tissue. Moreover, lower total cardiac FAs and SFA and higher cardiac n-6 PUFA were also associated with PPARγ activation. Altogether, there were characteristic effects of PPARα activation in liver and heart, as well as in plasma. PPARγ effects were not only confined to adipose tissue, but specific effects were also observed in liver, heart, and plasma. In conclusion, short-term treatment with PPAR agonists induced tissue-specific effects on FA composition in liver and heart. Moreover, both PPARα and PPARγ activation lowered plasma TAG and phospholipids, most likely through effects on liver and adipose tissue, respectively. In future studies we aim to reveal whether similar patterns can be found through diet-induced activation of specific pathways.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of nuclear receptors and are central transcription factors governing pathways involved in energy metabolism and homeostasis [1]. PPARα is highly expressed in metabolically active tissues like the liver, heart, and kidneys and is involved in regulating genes related to glucose and lipid metabolism [2], while PPARγ is abundantly expressed in adipose tissue, where it controls lipid storage and differentiation.
of adipocytes [3]. PPARγ is also expressed in several other tissues, and its action includes traits like improved insulin sensitivity [4] and holds anti-inflammatory properties [5].

Since PPARα has hypolipidemic and PPARγ has insulin sensitizing properties, these transcription factors have been of large clinical interest as targets for medical treatment of conditions involving cardiovascular disease and diabetes [6, 7]. Fatty acids (FAs) and their derivatives are natural ligands for the PPARs, and diet will consequently have a significant impact on their activities [8]. An increased focus on dietary fat, including the proposed beneficial effects of polyunsaturated FAs [9] and trend diets like the high fat-low carbohydrate diet [10], necessitates studies on how the internal organs are affected. Well-known synthetic PPAR ligands include fibrates activating PPARα and thiazolidinediones activating PPARγ [1]. Although the main metabolic effects of PPARα and γ have been documented in rodents and humans, few studies have performed such comprehensive and direct comparison of PPAR activation effects on lipid metabolism and lipid status across important metabolic organs.

The objective of the current study was to compare how short-term activation of PPARα and γ affected metabolism through lipid levels and FA composition, as well as gene regulation in liver and adipose tissue. For this purpose, male Wistar rats were treated for 12 days with the specific PPAR agonists WY-14,643 (PPARα) and rosiglitazone (PPARγ), followed by measuring hepatic and cardiac lipids, FA composition, as well as certain hepatic and adipose genes expected to respond to PPAR activation.

2. Methods

2.1. Animals and Study Design. The animal experiments were standardized according to the Guidelines for the Care and Use of Experimental Animals, and the protocol was approved by the Norwegian Committee for Experiments on Animals, and in accordance with the Norwegian legislation and regulations governing experiments using live animals (FOTS ID: 2014/6187). The experiment was done in accordance with the regulations laid down by the National Animal Research Authority.

A total of 20 male Wistar rats, aged eight weeks (200-225 g), were obtained from Taconic Europe A/S (Ry, Denmark). They were housed 2-3 animals per cage (Makrolon IV). The animal room maintained a constant 12 h light–dark cycle at a temperature of 22 ± 2°C, a relative humidity of 55 ± 5%, and underwent 20 air changes per hour. Common environmental enrichment was used. Animals were acclimatized under these conditions for 6 days prior to study start and had free access to standard chow and tap water throughout the study. During the following two days prior to study intervention, animals were habituated careful handling, and they were introduced to the muffin dough to be used as a vehicle for the PPAR agonists.

The animals were randomized into the following three groups: (1) Placebo (control, n=8); (2) PPARα agonist (n=6); and (3) PPARγ agonist (n=6). Block randomization was used when placing the animals into cages, as well as for the terminal operation. Sample size in each group was determined based on an assumed profound response (if any) in the PPAR intervention groups. All animals received a low-fat chow diet. In addition, during the 12 days of study intervention each animal was given a daily supplement of 300 µl muffin dough as a vehicle with or without agonist. Treatments were given according to the following daily routine: the placebo control group received pure vehicle (without agonist); the PPARα agonist group received vehicle with 20 mg/kg/day WY-14,643 (Tocris Bioscience, Bristol, UK); and the PPARγ agonist group received vehicle with 10 mg/kg/day rosiglitazone (Sigma-Aldrich, St. Louis, MO). Ingredients in the muffin dough vehicle were eggs, sugar, gluten free flour, vanilla sugar, milk, and butter. Animal weights were measured at day 0, day 6, and day 12 during the experiment. Feed intake was estimated by weighing the food provided to each cage and finally weighing the residual feed on day 12.

The animals received anesthesia with 2% isoflurane (Schering-Plough, Kent, UK) under fasting conditions after 12 days of intervention. The abdomen was opened in the midline and the animals were sacrificed by cardiac puncture and exsanguination. Blood was collected in BD Vacutainer tubes containing EDTA (Becton, Dickinson, and Company, Plymouth, UK). Liver, heart, and epididymal adipose tissue were collected, weighed, and snap-frozen as drainage of blood from the animal was complete. Liver mass relative to body weight (liver index) was calculated by the formula [100* (liver weight in g / body weight in g)]. A liver piece for the β-oxidation was collected by cutting a piece of the main lobe. A small piece was also collected for FA analyses. Plasma and tissue samples were stored at −80°C until analyses.

2.2. Biochemical Analyses. Plasma, hepatic, and cardiac lipids were measured on the Hitachi 917 system (Roche Diagnostics, GmbH, Mannheim, Germany). Total cholesterol, HDL cholesterol, LDL cholesterol, and TAG kits were from Roche Diagnostics and the phospholipids, free cholesterol, and free FAs kits from DiaSys Diagnostic Systems GmbH (Holzheim, Germany). Plasma carnitine metabolites were determined by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described [11–13]. FA methyl esters (FAME) were prepared from hepatic and cardiac tissues and analyzed by gas-liquid chromatography (GC) as previously described [14]. The anti-inflammatory index was calculated based on the formula [(C22:6n-3 + C22:5n-3 + C20:3n-6 + C20:5n-3) / C20:4n-6]*10 [15].

2.3. Hepatic Enzyme Activities. Liver tissue samples were homogenized and fractionated as previously described [16]. The postnuclear fraction was used for further analyses. Liquid scintillation with (1-14C) palmitoyl-CoA as a substrate was used to determine β-oxidation capacity in liver in the absence and presence of malonyl-CoA [17]. The activities of carnitine palmitoyl transferase 2 (CPT-II) [18], fatty acyl-CoA oxidase (ACOX) [19, 20], and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase [21] were measured as previously described.
2.4. Gene Expression Analyses. Total cellular RNA was purified from tissue using the RNaseasy kits with the RNaseasy® Mini protocol for liver and the RNaseasy® Lipid Tissue protocol for adipose tissue (Qiagen GmbH, Hilden, Germany). RNA quantity was determined spectrophotometrically (NanoDrop 1000, Thermo Scientific, Wilmington, DE, USA), while quality was evaluated by capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies Inc., Santa Clara, CA, USA). RNA was reversely transcribed to cDNA in 20 µl reactions (containing 500 mg RNA) using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Samples were treated with RNase inhibitors as part of the protocol. Selected genes were analyzed using qPCR with the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems): Rn00566193 (Ppara), Rn00440945 (Pparγ), Rn00580241 (Pgc1α), Rn00580702 (Cpt1a), Rn00563995 (Cpi2), Rn00571166 (Ucp2), Rn01460628 (Acodl), Rn00597399 (Hmgcs2), Rn00569117 (Fas), Rn00580728 (Cd36), Rn00664587 (Fabp1), Rn04219585 (Fabp4), Rn00558582 (Fatp1), Rn00561482 (Lpl), Rn04213343 (Pltp), Rn00561474 (Lipe), Rn00563444 (Lipe), Rn00562483 (ApoAI), Rn01499950 (ApoB), Rn01764530 (ApoC2), and Rn00560743 (ApoC3). All primer/probe sequences for the genes studied were obtained from Applied Biosystems. The MIQE guidelines for qPCR analyses were used when selecting house-keeping genes [22, 23]. Three house-keeping genes were tested: RT-CKFT-18s (house-keeping genes were tested: RT-CKFT-18s (Applied Biosystems), and Rn00821091 (Applied Biosystems). The house-keeping gene was found to be the best using NormFinder [24] and was used to normalize the expression value of each gene in all samples.

2.5. Statistical Analyses. All measurements except the gene expression data were log-transformed and presented as geometric means with their geometric standard deviations (gSD). Gene expression data was normalized against the control group (placebo) and presented as mean (SD) relative to the control group. The groups were compared by a one-way ANOVA, and the proportion of variance explained by the experimental groups was assessed by calculating the $\eta^2$. The assumption of equal variance was assessed with Levene's test and visually by plotting the residuals. Within-group normality was assessed visually by Q-Q plots of the residuals. Planned comparisons towards the control group were performed for the two intervention groups, and p-values were extracted from the regression model. Standardized mean difference (SMD; 95% confidence interval) were calculated and plotted to illustrate the differences from the control group. The p-values were adjusted using the method of Benjamini and Hochberg [25] to control the false discovery rate. The raw individual values were plotted with overlaying box plots. The data file was processed in IBM SPSS Statistics for Windows, version 23 (IBM Corporation, Armonk, NY, USA), and statistics were performed in R version 3.5.1 (https://www.R-project.org/), and the packages within the tidyverse (dplyr, broom, purr, magrittr, and rlang) and forestplot. P-values <0.05 were considered as statistically significant.

3. Results

3.1. Weights and Lipid Related Parameters. Details on weight measurements and lipid related parameters are illustrated in Figure 1. At study start geometric mean (gSD) weight of the animals was 247 (1.04) gram and during 12 days they gained 44.0 (1.22) gram. Neither baseline weights nor weight gain significantly differed between treatment groups. Of interest, the weights of the epididymal adipose tissue did not differ between groups treated with agonists, compared to control after 12 days intervention.

Treatment with WY-14,643 was associated with higher liver weight and liver index (SMD = 6.4 and 10.3), lower plasma total and HDL cholesterol (SMD = -5.2 and -4.5), triglycerides (TAG; SMD = -1.5), phospholipids (SMD = -3.6), and free cholesterol (SMD = -2.1), compared with control. Furthermore, animals receiving the PPARα agonist had lower plasma levels of the carnitine precursor butyrobetaine (SMD = -7.6), as well as carnitine (SMD = -1.6) and all measured acylcarnitines (SMD = -4.1 - 1.9).

In liver, PPARα activation was associated with lower total cholesterol (SMD = -2.0) and higher phospholipids (SMD = 2.0). β-oxidation was higher (SMD = 4.8), while the ability of inhibition by malonyl-CoA was substantially reduced (SMD = -2.9). The activities of carnitine palmitoyltransferase II (CPTII) (SMD = 5.4), fatty acyl-CoA oxidase (ACOX; SMD = 21.3), and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase (SMD = 3.7) were all higher following treatment with WY-14,643. Free cardiac FAs were lower following PPARα activation, compared with control (SMD = -1.4).

Treatment with rosiglitazone was associated with a lower liver index (SMD = -1.8), lower plasma TAG (SMD = -2.6) and phospholipids (SMD = -1.7), higher plasma butyrobetaine (SMD = 2.2), and higher hepatic phospholipids (SMD = 1.3). Supplemental Figure 1 illustrates the raw values of each measured parameter from Figure 1.

Feed intake was estimated throughout the study, and the rats who received the PPARγ agonist had a significantly higher intake of chow compared with control (P<0.001). However, there was no statistically significant impact on weight gain or feed efficiency (weight gain per feed intake (g)) in either of the groups.

3.2. Fatty Acid Composition in Liver and Heart. Overall, rats treated with the PPARα agonist had higher hepatic SFA (SMD = 2.5) and MUFA (SMD = 2.6) and lower n-6 (SMD = -1.8) and n-3 (SMD = -4.8) PUFAs, compared to control (Figure 2). Along the MUFA pathway there was a downstream increase towards a final higher mead acid (C20:3n-9; SMD = -7.6), a swella carnitine (SMD=-1.4) and a measured acylcarnitines (SMD = -4.1 - 1.9).

Overall, rats treated with the PPARα agonist had higher hepatic SFA (SMD = 2.5) and MUFA (SMD = 2.6) and lower n-6 (SMD = -1.8) and n-3 (SMD = -4.8) PUFAs, compared to control (Figure 2). Along the MUFA pathway there was a downstream increase towards a final higher mead acid (C20:3n-9; SMD = 9.3). PPARα activation was associated with lower hepatic content of the essential PUFAs linoleic (C18:2n-6) and α-linolenic (C18:3n-3) acids (SMD = -6.6 and -7.6), but with higher arachidonic acid (C20:4n-6; SMD = 1.5) and lower levels of the most downstream n-3 PUFAs (C20-22; SMD = -6.8, -5.8, and -3.2). Altogether, this was in line with a lower PUFA n-3/n-6 ratio (SMD = -3.3) and anti-inflammatory index (SMD = -2.6) after WY-14,643
|                         | Control       | PPARα        | PPARγ        | P ANOVA | η²  |
|-------------------------|---------------|--------------|--------------|---------|-----|
| Weight Day 0 (g)        | 248 (1.04)    | 252 (1.04)   | 240 (1.04)   | 0.158   | 20 %|
| Weight Day 6 (g)        | 272 (1.05)    | 274 (1.04)   | 267 (1.06)   | 0.592   | 6 % |
| Weight Day 12 (g)       | 292 (1.06)    | 292 (1.05)   | 290 (1.07)   | 0.969   | 0 % |
| Weight gain (g)         | 43.3 (1.24)   | 40.7 (1.10)  | 49 (1.25)    | 0.248   | 15 %|
| Liver weight (g)        | 8.84 (1.10)   | 14.3 (1.04)  | 8.03 (1.08)  | <0.001  | 93 %|
| Liver Index             | 3.03 (1.06)   | 4.89 (1.03)  | 2.77 (1.04)  | <0.001  | 97 %|
| Heart weight (g)        | 0.84 (1.11)   | 0.84 (1.05)  | 0.83 (1.09)  | 0.972   | 0 % |
| Adipose weight (g)      | 2.81 (1.36)   | 3.12 (1.19)  | 3.02 (1.22)  | 0.730   | 4 % |
| Cholesterol, mmol/L     | 1.56 (1.15)   | 0.74 (1.15)  | 1.36 (1.16)  | <0.001  | 85 %|
| HDL cholesterol, mmol/L | 1.19 (1.15)   | 0.40 (1.39)  | 1.1 (1.14)   | <0.001  | 86 %|
| LDL cholesterol, mmol/L | 0.14 (1.50)   | 0.11 (1.27)  | 0.20 (1.47)  | 0.027   | 35 %|
| Triglycerides, mmol/L   | 1.72 (1.34)   | 1.11 (1.36)  | 0.76 (1.40)  | 0.001   | 59 %|
| Phospholipids, mmol/L   | 1.77 (1.11)   | 1.23 (1.10)  | 1.49 (1.10)  | <0.001  | 74 %|
| Free cholesterol, mmol/L| 0.43 (1.21)   | 0.27 (1.27)  | 0.38 (1.23)  | 0.003   | 49 %|
| Free fatty acids, mmol/L| 0.25 (1.22)   | 0.21 (1.36)  | 0.26 (1.53)  | 0.445   | 9 % |
| Trimethyllysine, μmol/L | 1.74 (1.13)   | 1.75 (1.09)  | 1.77 (1.06)  | 0.959   | 0 % |
| Butyrobetaine, μmol/L   | 0.901 (1.08)  | 0.518 (1.07) | 1.08 (1.09)  | <0.001  | 95 %|
| Carnitine, μmol/L       | 41.3 (1.11)   | 31.8 (1.25)  | 40.3 (1.23)  | 0.031   | 34 %|
| Acetylcarnitine, μmol/L | 21 (1.19)     | 14 (1.28)    | 26.2 (1.33)  | 0.001   | 57 %|
| Octanoylcarnitine, μmol/L| 0.014 (1.08) | 0.010 (1.15) | 0.015 (1.20) | <0.001  | 64 %|
| Palmitoylcarnitine, μmol/L| 0.15 (1.24) | 0.07 (1.13)  | 0.15 (1.36)  | <0.001  | 72 %|
| Propionylcarnitine, μmol/L| 0.75 (1.19) | 0.31 (1.38)  | 0.84 (1.27)  | <0.001  | 79 %|
| (Iso)Valerylcarnitine, μmol/L| 0.18 (1.19) | 0.09 (1.20)  | 0.21 (1.21)  | <0.001  | 83 %|
| Liver Cholesterol, μmol/g| 6.62 (1.06)  | 5.92 (1.05)  | 6.78 (1.09)  | 0.005   | 47 %|
| Liver triglycerides, μmol/g| 6.8 (1.21)   | 5.68 (1.09)  | 5.89 (1.40)  | 0.300   | 13 %|
| Liver phospholipids, μmol/g| 24.6 (1.07) | 27.7 (1.05)  | 27 (1.08)    | 0.008   | 44 %|
| Beta−oxidation, , mmol/mg/min| 0.19 (1.18)  | 0.66 (1.39)  | 0.22 (1.36)  | <0.001  | 83 %|
| Inhibition of Beta−oxidation by Malonyl−CoA, %| 65.1 (1.08)  | 19.8 (1.79)  | 51.3 (1.32)  | <0.001  | 69 %|
| CPTII, nmol/mg/min       | 0.65 (1.70)   | 8.03 (1.46)  | 1.07 (1.44)  | <0.001  | 88 %|
| ACOX, nmol/mg/min        | 8.06 (1.13)   | 102 (1.12)   | 9.2 (1.12)   | <0.001  | 99 %|
| HMG-CoA, nmol/mg/min     | 1.5 (1.32)    | 8.98 (1.85)  | 1.45 (1.22)  | <0.001  | 84 %|
| Heart Cholesterol, μmol/g| 3.84 (1.05)   | 3.7 (1.02)   | 3.75 (1.04)  | 0.177   | 18 %|
| Heart triglycerides, μmol/g| 3.08 (2.92)  | 1.53 (1.29)  | 1.34 (1.19)  | 0.085   | 25 %|
| Heart phospholipids, μmol/g| 13.2 (1.06)  | 13.8 (1.03)  | 13 (1.05)    | 0.112   | 23 %|
| Heart Free fatty acids, μmol/g| 0.25 (1.30) | 0.18 (1.17)  | 0.21 (1.12)  | 0.029   | 34 %|

Figure 1: Weights and lipid related parameters in Wistar rats during treatment with PPAR agonists for 12 days. Values are geometric mean (gSD). Red bars correspond to PPARα vs control and blue bars to PPARγ vs control. Measurements of variables are in plasma, unless otherwise stated. ACOX, fatty acyl-CoA oxidase; ANOVA, analysis of variance; CPTII, carnitine palmitoyltransferase II; EPI, epididymal fat; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A synthase; LDL, low density lipoprotein; PPAR, peroxisome proliferator-activated receptor.
Figure 2: Hepatic fatty acid composition (wt%) in Wistar rats after treatment with PPAR agonists for 12 days. Values are geometric mean (gSD). Red bars correspond to PPARα vs control and blue bars to PPARγ vs control. ANOVA, analysis of variance; MUFA, monounsaturated fatty acids; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. D5 desaturase index (n-6) = C20:4n-6/C20:3n-6 (an indirect index of Δ5 desaturase activity based on n-6 PUFA). D5 desaturase index (n-3) = C20:5n-3/C20:4n-3 (an indirect index of Δ5 desaturase activity based on n-3 PUFA). D6 desaturase index (n-6) = C18:3n-6/C18:2n-6 (an indirect index of Δ6 desaturase activity based on n-6 PUFA). D6 desaturase index (n-3) = C18:4n-3/C18:3n-3 (an indirect index of Δ6 desaturase activity based on n-3 PUFA). D9 desaturase index (C16) = C16:1n-7/C16:0 (an indirect index of Δ9 desaturase activity based on C16 SFA/MUFA). D9 desaturase index (C18) = C18:1n-9/C18:0 (an indirect index of Δ9 desaturase activity based on C18 SFA/MUFA). Anti-inflammatory index = ((C22:6n-3 + C22:5n-3 + C20:5n-3 + C20:3n-6 + C20:4n-6) / C20:4n-6) * 100.

treatment. Furthermore, there was an overall lower estimated Δ5 desaturase activity and a higher estimated Δ6 desaturase activity in animals treated with the PPARα agonist. The Δ9 desaturase index based on C16 was lower (SMD = -3.1), while that based on C18 was higher (SMD = 4.1).

The strongest effects on hepatic FA composition following treatment with rosiglitazone were higher contents of mead acid (C20:3n-9; SMD = 3.6) and eicosapentaenoic acid (C20:5n-3; SMD = 2.7) compared to control, the last mentioned also illustrated by the increase in estimated Δ5 desaturase activity along the n-3 pathway (SMD = 1.9).

Supplemental Figure 2 illustrates the raw values of each measured parameter from Figure 2. FA composition in plasma was very similar to that observed in hepatic tissue, with few exceptions (Supplemental Figure 3).

Certain changes were also seen in heart tissue following treatment with the PPAR agonists (Figure 3). Administration of WY-14,643 was associated with higher mead acid
|                          | Control          | PPARα            | PPARγ            | P ANOVA | $\eta^2$ |
|--------------------------|------------------|------------------|------------------|---------|---------|
| Total fatty acids, µg/g tissue | 22700 (1.11) | 22000 (1.05) | 20200 (1.05) | 0.024  | 36 %    |
| SFA                      | 30.6 (1.04)     | 30.3 (1.02)     | 29.1 (1.01)     | 0.007  | 44 %    |
| C16:0                    | 12.1 (1.22)     | 11.1 (1.03)     | 10.9 (1.04)     | 0.289  | 14 %    |
| C18:0                    | 16.6 (1.13)     | 17.9 (1.04)     | 16.6 (1.04)     | 0.227  | 16 %    |
| MUFA                     | 10.4 (1.48)     | 7.49 (1.17)     | 8.69 (1.10)     | 0.106  | 23 %    |
| C16:1 n−7                | 0.69 (2.50)     | 0.26 (1.29)     | 0.44 (1.22)     | 0.032  | 33 %    |
| C18:1 n−7                | 3.52 (1.08)     | 2.85 (1.06)     | 4.08 (1.14)     | <0.001 | 73 %    |
| C16:1 n−9                | 0.068 (1.44)    | 0.093 (1.14)    | 0.059 (1.12)    | 0.018  | 38 %    |
| C18:1 n−9                | 5.05 (1.81)     | 3.7 (1.29)      | 3.56 (1.09)     | 0.232  | 16 %    |
| C20:3 n−9                | 0.11 (1.12)     | 0.26 (1.29)     | 0.14 (1.09)     | <0.001 | 86 %    |
| PUFA n−6                 | 43.1 (1.09)     | 43.8 (1.06)     | 48.1 (1.02)     | 0.011  | 41 %    |
| C18:2 n−6                | 24.3 (1.08)     | 19.9 (1.20)     | 29.2 (1.06)     | <0.001 | 67 %    |
| C20:4 n−6                | 16.5 (1.15)     | 21.5 (1.07)     | 16.7 (1.09)     | 0.001  | 57 %    |
| PUFA n−3                 | 14.6 (1.20)     | 17.7 (1.21)     | 13.8 (1.11)     | 0.043  | 31 %    |
| C18:3 n−3                | 0.23 (2.04)     | 0.14 (1.21)     | 0.16 (1.11)     | 0.138  | 21 %    |
| C18:4 n−3                | 0.0019 (4.26)   | 0.0003 (1.80)   | 0.0008 (2.94)   | 0.026  | 35 %    |
| C20:5 n−3                | 0.25 (1.15)     | 0.20 (1.20)     | 0.30 (1.17)     | 0.001  | 55 %    |
| C22:5 n−3                | 1.85 (1.18)     | 15 (1.22)       | 11.6 (1.12)     | 0.041  | 31 %    |
| C22:6 n−3                | 12.1 (1.25)     | 15.3 (1.22)     | 11.6 (1.12)     | 0.171  | 19 %    |
| PUFA n−3/PUFA n−6 ratio  | 0.34 (1.13)     | 0.41 (1.27)     | 0.29 (1.13)     | 0.009  | 42 %    |
| D5 desaturase index (n−6)| 31.7 (1.19)     | 32.5 (1.16)     | 26.9 (1.12)     | 0.075  | 26 %    |
| D5 desaturase index (n−3)| 6.57 (1.16)     | 4.73 (1.24)     | 6.61 (1.21)     | 0.006  | 45 %    |
| D6 desaturase index (n−6)| 0.0014 (1.41)   | 0.0018 (1.12)   | 0.0012 (1.12)   | 0.040  | 31 %    |
| D6 desaturase index (n−3)| 0.0084 (2.83)   | 0.0022 (1.85)   | 0.0047 (3.11)   | 0.044  | 31 %    |
| D9 desaturase index (C16) | 0.057 (2.06)    | 0.024 (1.28)    | 0.040 (1.18)    | 0.015  | 39 %    |
| D9 desaturase index (C18) | 0.30 (2.04)     | 0.21 (1.34)     | 0.21 (1.12)     | 0.287  | 14 %    |
| Anti-inflammatory index   | 89.4 (1.07)     | 84.8 (1.15)     | 85 (1.08)       | 0.518  | 7 %     |
Cluster of differentiation 36 (CD36) into ketone bodies. Transport proteins important for FAs include fatty acid transport protein 1 (FABP1), fatty acid binding protein 1 (FABP1), apolipoprotein A1 (APOA1), apolipoprotein B (APOB), apolipoprotein C2 (APOC2), apolipoprotein C3 (APOC3), CD36, cluster of differentiation 36; CPT1a, carnitine palmitoyltransferase 1a; CPT2, carnitine palmitoyltransferase 2; Fabp1, fatty acid binding protein 1; Fas, fatty acid synthase; Fatp1, apolipoprotein A1; ApoB, apolipoprotein B; ApoC2, apolipoprotein C2; ApoC3, apolipoprotein C3; Cd36, cluster of differentiation 36; Hmgcs2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; Lpl, lipoprotein lipase; Pgc1alpha, peroxisome proliferator-activated receptor γ coactivator 1α; Pltp, phospholipid transfer protein; Pparα, peroxisome proliferator-activated receptor α; Pparγ, peroxisome proliferator-activated receptor γ; TNFα, tumor necrosis factor alpha; Ucp2, uncoupling protein 2.

**Figure 4**: Hepatic gene expression in Wistar rats after treatment with PPAR agonists for 12 days, normalized towards the control group. Red bars correspond to PPARα vs control and blue bars to PPARγ vs control. Acox1, fatty acyl-CoA oxidase 1; ANOVA, analysis of variance; ApoAI, apolipoprotein A1; ApoB, apolipoprotein B; ApoC2, apolipoprotein C2; ApoC3, apolipoprotein C3; Cd36, cluster of differentiation 36; Cpt1a, carnitine palmitoyltransferase 1a; Cpt2, carnitine palmitoyltransferase 2; Fabp1, fatty acid binding protein 1; Fas, fatty acid synthase; Fatp1, fatty acid transport protein 1; Hmgcs2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; Lpl, hepatic lipase C; Lpl, lipoprotein lipase; Pgc1alpha, peroxisome proliferator-activated receptor γ coactivator 1α; Pltp, phospholipid transfer protein; Pparα, peroxisome proliferator-activated receptor α; Pparγ, peroxisome proliferator-activated receptor γ; TNFα, tumor necrosis factor alpha; Ucp2, uncoupling protein 2.

Supplemental Figure 5 illustrates the raw values of gene expression relative to control from Figure 4.

**4. Discussion**

Overall, in the current short-term study of PPARα and PPARγ activation, we present a comprehensive report with specific effects on circulating lipids, FA composition in liver, heart, and plasma and expression of known PPAR target genes in liver and adipose tissue. PPARα activation was obtained by treatment with WY-14,643, while PPARγ activation was obtained by treatment with rosiglitazone. PPARα activation was associated with lower plasma total cholesterol, HDL cholesterol, TAG, and phospholipids, higher hepatic phospholipids, SFA and MUFA, lower hepatic n-6 and n-3.
| Gene       | Control       | PPARα        | PPARγ        | P ANOVA | $\eta^2$ |
|------------|---------------|--------------|--------------|---------|----------|
| PPARα      | 1.00 (0.26)   | 0.84 (0.08)  | 1.32 (0.40)  | 0.024   | 36 %     |
| PPARγ      | 1.00 (0.36)   | 0.84 (0.34)  | 1.10 (0.46)  | 0.510   | 8 %      |
| Pgc1α      | 1.00 (0.43)   | 0.85 (0.23)  | 1.94 (1.14)  | 0.027   | 35 %     |
| Cpt1α      | 1.00 (0.21)   | 0.94 (0.25)  | 0.88 (0.19)  | 0.592   | 6 %      |
| Cpt2       | 1.00 (0.29)   | 0.89 (0.08)  | 2.53 (0.83)  | <0.001  | 72 %     |
| Ucp2       | 1.00 (0.36)   | 0.84 (0.11)  | 1.03 (0.36)  | 0.508   | 8 %      |
| Acox1      | 1.00 (0.26)   | 0.95 (0.11)  | 1.71 (0.53)  | 0.001   | 54 %     |
| Hmgcs2     | 1.00 (0.09)   | 1.23 (0.47)  | 0.90 (0.32)  | 0.198   | 17 %     |
| Fas        | 1.00 (0.62)   | 0.89 (0.45)  | 1.64 (0.73)  | 0.099   | 24 %     |
| Cd36       | 1.00 (0.31)   | 0.98 (0.24)  | 1.42 (0.49)  | 0.076   | 26 %     |
| Fabp4      | 1.00 (0.32)   | 0.92 (0.17)  | 1.55 (0.57)  | 0.021   | 37 %     |
| Fatp1      | 1.00 (0.41)   | 0.85 (0.32)  | 3.57 (1.46)  | <0.001  | 70 %     |
| Lpl        | 1.00 (0.31)   | 0.90 (0.20)  | 0.85 (0.27)  | 0.590   | 6 %      |
| Pltp       | 1.00 (0.23)   | 1.18 (0.14)  | 0.86 (0.23)  | 0.048   | 30 %     |
| Lipe       | 1.00 (0.37)   | 0.79 (0.20)  | 0.60 (0.33)  | 0.096   | 24 %     |
| TNFα       | 1.00 (0.26)   | 1.11 (0.61)  | 0.81 (0.47)  | 0.517   | 7 %      |

**Figure 5:** Epididymal adipose tissue gene expression in Wistar rats after treatment with PPAR agonists for 12 days, normalized towards the control group. Red bars correspond to PPARα vs control and blue bars to PPARγ vs control. Acx, fatty acyl-CoA oxidase 1; ANOVA, analysis of variance; Cd36, cluster of differentiation 36; Cpt1α, carnitine palmitoyltransferase 1α; Cpt2, carnitine palmitoyltransferase 2; Fabp4, fatty acid binding protein 4; Fas, fatty acid synthase; Fatp1, fatty acid transport protein 1; Hmgcs2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; Lipe, hormone sensitive lipase E; Lpl, lipoprotein lipase; Pgc1α, peroxisome proliferator-activated receptor α coactivator 1α; Pltp, phospholipid transfer protein; Pparα, peroxisome proliferator-activated receptor α; Pparγ, peroxisome proliferator-activated receptor γ; TNFα, tumor necrosis factor alpha; Ucp2, Uncoupling protein 2.

**Figure 6:** A summary showing main effects of PPARα and PPARγ activation as observed in the current study in the circulation, liver, heart, and adipose tissue as indicated. PPARα effects are shown in red, while PPARγ effects are shown in blue.
PUFA, and higher cardiac arachidonic acid. PPAR\(\gamma\) activation was associated with lower plasma TAG and phospholipids, lower cardiac total FAs and SFA, and higher cardiac n-6 PUFA. A summary of characteristic effects of PPAR\(\alpha\) and PPAR\(\gamma\) activation in the circulation, as well as in liver, heart, and adipose tissue is illustrated in Figure 6.

The effects of PPAR\(\alpha\) agonist stimulation on hepatic lipid metabolism in rodents are well-documented and include induced FA uptake, \(\beta\)-oxidation, ketogenesis, and TAG clearance [26]. Similar effects are observed in heart and skeletal muscle, but to a much lesser degree due to the tissue-specific expression pattern of PPAR\(\alpha\) [27]. Thus, liver plays a major role in PPAR\(\alpha\)-induced plasma lipid lowering. Moreover, hepatic peroxisome proliferation, related to increased liver weight, is a well-known effect in response to PPAR stimulation in rodents [28]. In addition, activation of PPAR\(\alpha\) will result in body weight loss after prolonged treatment [29, 30]. PPAR\(\gamma\) agonists, in contrast, have more prominent effects on adipose tissue. Rosiglitazone has been shown to stimulate the lipid storage capacity of adipose tissue, increase lipid uptake, and efficiently reduce plasma glucose levels [31–33].

Activation of PPAR\(\gamma\) has been postulated to regulate the expression of adipose triglyceride lipase (ATGL), which has an important role in lipid metabolism [34]. Furthermore, rosiglitazone treatment has been associated with increased ATGL expression in WAT and BAT of lean and obese mice [34].

Although the roles of PPAR\(\alpha\) and \(\gamma\) on lipid metabolism in rodents have been described previously, few studies have performed a comprehensive comparison of PPAR\(\alpha\) and PPAR\(\gamma\) agonists on lipids, FA composition, and gene expression in major organs involved in lipid turnover. Findings in animals treated with the PPAR\(\alpha\) agonist indicated an increased hepatic \(\beta\)-oxidation and ketogenesis compared to rats treated with vehicle. An enhanced FA transport and uptake is supported by an increased hepatic expression of Cld\(3\_6\), Fabp\(1\), and Fatp\(1\) [26]. Interestingly, gene expression in adipose tissue was not influenced by two weeks of WY-14,643 treatment. Rosiglitazone treatment led to higher expression of hepatic Pgc\(1\), Cpt\(1\), and Cld\(3\_6\), although not to an extent comparable with the effect of WY-14,643 treatment. Moreover, pharmacological activation of PPAR\(\gamma\) was associated with higher expression of adipose Cpt\(2\), Accox\(1\), Fabp\(4\), and Fatp\(1\) compared with control. As there was a strong plasma TAG reducing effect after PPAR\(\gamma\) activation, our results indicate that PPAR\(\gamma\)-induced lipid transport and catabolism in adipose tissue may contribute to the reduction in plasma TAG levels. Similarly, short-term PPAR\(\alpha\) activation, through rosiglitazone therapy, showed reduced plasma TAG and NEFAs in Wistar rats [35]. Moreover, Harrington et al. showed that a PPAR\(\gamma\) agonist GW7845 reduced plasma TAG levels in AKR/J mice treated for four weeks, which did not relate to increased in vitro hepatic FA oxidation [36]. PPAR\(\gamma\) activation has previously shown to reduce plasma TAG in rats through adipose tissue-specific increase in LPL activity as well as increased gene expression of FA transport proteins, and this process requires mTOR activity [37, 38]. Increased LPL activity has been associated with enhanced lipolytic activity, which in turn is associated with TAG clearance [39].

In the current study, gene expression patterns demonstrated a minor decrease in Lpl in adipose tissue following treatment with both PPAR agonists. On the contrary, there was an enhanced activity of genes associated with \(\beta\)-oxidation in adipose tissue following rosiglitazone treatment.

Several studies have shown that PPAR\(\gamma\) agonists increase feed intake and body weight and reduce plasma glucose and insulin levels, while the PPAR\(\alpha\) agonists reduce body weight [36]. We did not observe any statistically significant difference in weight gain between the treatment groups, despite an increase in feed intake among PPAR\(\gamma\) supplemented rats. Moreover, glucose levels were unchanged. This could be due to the relatively short treatment period, as the PPAR\(\gamma\) group did gain more weight in absolute terms.

Plasma carnitine metabolites were in general reduced after treatment with WY-14,643, but there were minor effects after treatment with rosiglitazone. Treatment of rats with a PPAR\(\alpha\) specific agonist [40] and a pan PPAR agonist with main affinity for PPAR\(\alpha\) [41] did also demonstrate reduced plasma acylcarnitine levels and increased expression of the Bbox\(1\) gene involved in production of the carnitine precursor butyrobetaine. Carnitine is essential for the transport of medium and long fatty acyl chains in and out of the mitochondrion, and plasma levels may reflect intracellular levels. Thus, the reduction in plasma carnitine and its precursor butyrobetaine by PPAR\(\gamma\) may have been due to an increased utilization of carnitine for FA transport, while the reduction in acylcarnitines could be linked to the increased \(\beta\)-oxidation and ketogenesis, lowering the levels of intermediate and end-products of \(\beta\)-oxidation. In line with this, acylcarnitines have been proposed as sensors of mitochondrial FA oxidation [42], and high levels of palmitoylcarnitine and octanoylcarnitine are linked to poor prognosis in patients with cardiovascular disease [13, 43]. Our results indicate that PPAR\(\gamma\) has little influence on plasma acylcarnitines, despite the increased expression of genes involved in adipose tissue \(\beta\)-oxidation.

Hepatic peroxisome proliferation leads to induced peroxisomal- and as an indirect consequence also mitochondrial \(\beta\)-oxidation [44]. This strong effect on liver exerted by PPAR\(\alpha\) may be reflected by its impact on hepatic FA composition. Although peroxisome proliferation does not occur in a similar manner in humans as in rodents, changes in FA composition in relation to \(\beta\)-oxidation rate can provide clues in the search for possible FA markers indicating PPAR activation under certain circumstances also in humans. It is conceivable that an increased peroxisomal catabolism of very long-chain FAs and subsequent oxidation in the mitochondria may induce shifts in FA composition. A study in ageing rats indicated that extent of peroxisomal \(\beta\)-oxidation affected brain FA composition [44]. Moreover, WY-14643 affected FA composition of myocardial phospholipids [45]. We have previously studied long-term PPAR\(\alpha\) activation in rats, resulting in elevated cardiac levels, as opposed to lower hepatic levels of n-3 PUFAs [14]. These tissue-specific differences are similar to those observed in the current short-term study, including lower hepatic n-3 PUFA and a tendency of elevated cardiac n-3 PUFA levels. The
lower hepatic level of n-3 PUFAs in animals treated with PPARα agonist may be a consequence of the increased hepatic β-oxidation, particularly since the long-chain n-3 PUFAs are preferred FA substrates for β-oxidation [46]. Interestingly, in both studies, mead acid was elevated in liver and heart, a PPAR mechanism which may be linked with an essential FA deficiency [47]. It is not straightforward to interpret exactly which mechanisms can be related to levels of SFAs and MUFAs, as these are more dependent on endogenous conversion compared to those of the PUFA subtype which are more directly related to the dietary intake [48].

In the current study, focus has been on PPARα- and γ-specific effects in metabolic active tissues, including liver, heart, and adipose tissue. Altogether, induced β-oxidation and enzymatic activity of PPARα target proteins were supportive of well-known PPARα specific effects in liver. Short-term treatment with rosiglitazone, a PPARγ specific synthetic agonist, reduced plasma TAG in male Wistar rats, which may relate to adipose tissue-specific effects on mitochondrial function and lipid uptake. Moreover, PPARγ activity seemed to affect β-oxidation in adipose tissue. Altogether, the current study demonstrates that PPARα and PPARγ specific ligands will influence lipids, gene expression, as well as FA composition in a tissue-specific manner. These findings are important for future studies on dietary components, when investigating which traits can be associated with PPAR related effects. It is also interesting to reveal PPAR effects on carnitine metabolites, which are potential biomarkers in human disease [49].

5. Conclusions

Short-term treatment with synthetic PPARα and PPARγ agonists induced changes in circulating lipids and FA composition in liver and heart, modifying mitochondrial function in a tissue-specific manner. Interestingly, we observed TAG and phospholipid lowering effects in plasma after treatment with both agonists. The ultimate future aim is to gain knowledge on how these parameters may be affected not only by activation through specific PPAR agonists, but also by dietary FAs as well as other dietary and lifestyle related factors. More knowledge regarding PPAR activity may be a part of the puzzle when laying the foundation for patient-specific dietary and medical prevention and treatment of metabolic diseases like obesity, diabetes, and cardiovascular diseases.

Data Availability

The SPSS data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Preliminary results from the current study were presented as a poster entitled “Short-term activation of PPARs, which are important facilitators in fatty acid metabolism, induce changes in hepatic and cardiac fatty acid composition in rats” by M. L. Grinna, B. Bjørndal, P. Bohov, R. Berge, O. Nygård, and E. Strand at “The 11th Nordic Nutrition Conference NNC2016” in Gothenburg, Sweden, June 20.-22. 2016.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors are grateful to Torunn Eide, Svein Krüger, Kari Mortensen, Randi Sandvik, Marte Trollebø, Kari Williams, and Liv Kristine Øysæd for valuable technical assistance. We also thank the staff at the University of Bergen lab animal facility for their important contributions during the animal experiments. This work was funded by grants from the Bergen Research Foundation.

Supplementary Materials

Supplemental Figure 1: individual data on weights and lipid related parameters in Wistar rats during treatment with PPAR agonists for 12 days. Supplemental Figure 2: individual data on hepatic fatty acid composition (wt%) in Wistar rats after treatment with PPAR agonists for 12 days. Supplemental Figure 3: plasma fatty acid composition (wt%) illustrated as (A) lines indicating geometric mean (gSD) values and as (B) individual raw data on plasma fatty acid composition (wt%) in Wistar rats after treatment with PPAR agonists for 12 days. Red bars correspond to PPARα vs control and blue bars to PPARγ vs control. ANOVA, analysis of variance; MUFA, monounsaturated fatty acids; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Supplemental Figure 4: individual data on cardiac fatty acid composition (wt%) in Wistar rats after treatment with PPAR agonists for 12 days. Supplemental Figure 5: individual hepatic gene expression, normalized towards the control group. Supplemental Figure 6: individual epididymal adipose tissue gene expression, normalized towards the control group. (Supplementary Materials)

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