Peroxisome proliferator-activated receptor alpha (PPARα) plays a crucial role in the transcriptional regulation of myocardial lipid metabolism. In vitro studies on isolated cardiomyocytes showed that PPARα activation induces expression of numerous genes involved in virtually all steps of fatty acid catabolism. However, there is very few data on the effect of PPARα activation on the content and composition of myocardial lipids in vivo. Therefore, our main aim was to examine effects of selective PPARα agonist WY-14643 on the content and fatty acid composition of major lipid classes in the heart of rats fed a standard chow (STD) or a high-fat diet (HFD). In STD rats WY-14643 paradoxically decreased palmitate oxidation rate in the heart, however, in HFD animals such effect was not observed. WY-14643 markedly reduced myocardial free fatty acid and diacylglycerol content in STD rats, whereas in HFD group the opposite effect was observed. These changes reflected alterations in plasma lipid concentration which suggests that effects of WY-14643 on the heart were indirect and secondary to changes in plasma lipid availability induced by the drug. Basal myocardial glucose uptake was not affected by PPARα agonist in either group, however, glycogen content in the heart was markedly increased. WY-14643 exerted profound influence on the fatty acid composition of myocardial phospholipids in both diet groups. These changes included increased percentage of monounsaturated fatty acids and replacement of n-3 polyunsaturated fatty acids (PUFA) by those from the n-6 family. This action of WY-14643 might be detrimental to the heart since n-3 PUFA possess cardioprotective and antiarrhythmic properties.

Keywords: PPAR, lipids, myocardium, high-fat diet, diacylglycerol, docosahexaenoic acid, arachidonic acid, fibrates

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the large superfamily of nuclear receptors. Their endogenous agonists include a variety of long-chain fatty acids and fatty acid derivatives such as prostaglandins and leukotrienes. There are three major PPAR isoforms termed α, δ and γ which all form heterodimers with retinoid X receptor (RXR) and bind to PPAR responsive element (PPRE) found in the promoter region of various genes involved in lipid metabolism and energy homeostasis (17). PPARα is expressed at a high level in tissues exhibiting high rates of fatty acid β-oxidation including liver, heart and kidney (12). In the recent years, the fibrate class of hypolipidemic drugs which is widely used in patients with metabolic syndrome has been shown to function through direct PPARα activation (14).

PPARα plays a pivotal role in the transcriptional regulation of lipid metabolism in the heart. In vitro studies on isolated rodent cardiomyocytes showed that activation of this receptor induces expression of a number of genes encoding proteins involved in virtually all steps of fatty acid metabolism. These steps include: fatty acid uptake (fatty acid transport protein, fatty acid translocase/CD36) and activation (acyl-CoA synthetase), transport of acyl-CoA to mitochondrial matrix (carnitine palmitoyltransferase (CPT) 1 and 2), mitochondrial β-oxidation (short, medium, long and very long-chain acyl-CoA dehydrogenases, short-chain 3-hydroxyacyl-CoA dehydrogenase, 3-ketoacyl-CoA thiolase) and peroxisomal β-oxidation (acyl-CoA oxidase) (4). In addition, PPARα activation induces expression of malonyl-CoA decarboxylase which is the enzyme responsible for degradation of malonyl-CoA – a competitive inhibitor of CPT1 playing an important role in the regulation of mitochondrial fatty acid oxidation in the heart (5). Consistently, PPARα knockout mice exhibit considerably lower myocardial fatty acid oxidation rate and expression of related enzymes (33). The heart of these animals is also unable to adapt to increased fatty acid availability resulting from fasting or high-fat feeding (11, 18). On the other hand, cardiac-specific overexpression of PPARα leads to increased fatty acid utilization in the heart (15).

Over the last decade the role of PPARα in the regulation of myocardial lipid metabolism was intensively studied. However, the vast majority of available papers focused on changes in expression of PPARα-regulated genes and there is very few data on the effect of PPARα agonists on the content and composition of myocardial lipids. Therefore, considering the importance of lipids in the membrane function and cellular signaling, our main aim was to examine effects of selective PPARα agonist – WY-
14643 on the content and fatty acid composition of major lipid classes in the rat heart.

MATERIALS AND METHODS

Animals and study design

The investigation was approved by the Ethical Committee for Animal Experiments at the Medical University of Bialystok. Male Wistar rats (200-240g) were housed in separate cages under controlled conditions (22°C ± 2, 12h light/12h dark cycle) with unlimited access to water. The animals were divided into two groups: 1) fed ad libitum on a standard laboratory rat chow (Agropol, Motycz, Poland) containing 2.8% of fat by weight and 2) fed for three weeks on a high-fat diet containing 33.9% of fat (sunflower oil) by weight, prepared as described by Pascoe and Storlien (28). The high-fat diet was given daily in the amount of 0.01% butylated hydroxytoluene (Sigma) as an antioxidant.

Animals and study design

Animals were anesthetized by intraperitoneal injection of pentobarbital in a dose of 80 mg/kg of body weight. Samples of the left ventricle were excised, cleaned of the blood and immediately freeze-clamped with aluminum tongs precooled in liquid nitrogen and then stored at -80°C until analysis. All samples were collected between 8 and 10 am.

Content of myocardial lipids

The samples of the myocardium were pulverized in an aluminum mortar precooled in liquid nitrogen. The powder was then transferred to a tube containing ice-cold methanol and 0.01% butylated hydroxytoluene (Sigma) as an antioxidant. Lipids were extracted by the method of Folch. The fractions of total phospholipids (PH), triacylglycerols (TAG), diacylglycerols (DAG) and free fatty acids (FFA) were separated by thin-layer chromatography (TLC) according to Roemen and van der Vusse (29). Lipid class standards were spotted on the outside lanes of the TLC plate to enable localization of the sample lipid classes. The gel bands corresponding to the standards were scrapped off the plates, transferred into fresh tubes and then transmethylated in 14% methanolic boron trifluoride (Sigma) at 100°C for either 2 (FFA), 10 (DAG) or 30 minutes (PH and TAG). The content of resulting fatty acid methyl esters was determined by means of gas-liquid chromatography as previously described in detail (25). Plasma FFA composition was determined using the same method as for myocardial FFA. Plasma TAG concentration was measured with the Serum Triglyceride Determination Kit (Sigma).

Glycogen content

The content of glycogen in the heart was determined following digestion of the samples in 30% KOH at 100°C for 20 min. Glycogen was then precipitated with ethanol from an aliquot of the digest and assayed by the anthrone method of Carroll et al. (6).

2-deoxyglucose uptake by the heart

The uptake of 2-deoxyglucose was determined as described by Turinsky et al. (31), with the exception that [1-14C]sucrose instead of [14C]sucrose was used to measure the extracellular space. The rats were anesthetized and 1 μCi of 2-deoxy-D-[1-14C]glucose (specific activity 56 mCi/mmol, NEN Life Science Products) and 5 μCi of [1-3H]mannitol (specific activity 26.3 mCi/mmol, NEN Life Science Products) per 100g body weight were administered into the tail vein. Samples of the cardiac muscle and the blood were taken 25 min later. The samples were then digested in 1M NaOH followed by neutralization with 1M HCl. The scintillation cocktail (Ultima Gold, Packard) was added and the radioactivity was counted. The 2-deoxyglucose uptake was calculated as the difference between the total myocardial radioactivity and the radioactivity of the myocardial extracellular space.

Estimation of palmitate oxidation rate in the heart

The rats were anesthetized and 5μCi of [1-14C]palmitate (specific activity 55 mCi/mmol, PerkinElmer) bound to albumin per 100g body weight was injected into the tail vein. Samples of the heart’s left ventricle and blood were taken 10 minutes after administration of the radiolabeled palmitate. The rate of myocardial palmitate oxidation was estimated from the radioactivity of the acid-soluble metabolites of [14C]palmitate (acetyl-CoA, acetylcarnitine and citric acid cycle intermediates) determined as described by Demaison et al. (10). The index of cardiac palmitate oxidation (Rpn) was calculated according to Oakes et al. (27):

\[ R_{pn} = C_{FA} \times \frac{m_B}{m_A} \]

where \( C_{FA} \) is the plasma FFA concentration, \( m_B \) is the tissue content of acid-soluble products of [14C]palmitate oxidation and \( C_A \) is the plasma concentration of free [14C]palmitate. Total plasma FFA concentration was determined using the Wako NEFA C kit (Wako Chemicals). In order to measure the content of plasma free [14C]palmitate plasma lipids were extracted and separated by means of TLC as described above. Radioactivity of the gel band corresponding to the FFA standard was then counted.

Myocardial expression of PPARα and β

The analysis of the content of PPARα, and β protein was carried out with the use of Western blot technique. Briefly, protein extracts (25 μg) were fractionated on a 10% polyacrylamide gel, transferred to nitrocellulose membranes, and probed with the

Table 1. Fatty acid composition of the experimental diets.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Standard chow</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (14:0)</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>21.8</td>
<td>8.1</td>
</tr>
<tr>
<td>Palmitoleic (16:1, n-7)</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>12.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Oleic (18:1, n-9)</td>
<td>20</td>
<td>28.1</td>
</tr>
<tr>
<td>Linoleic (18:2, n-6)</td>
<td>35.5</td>
<td>57.6</td>
</tr>
<tr>
<td>Arachidonic (20:4, n-6)</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>α-Linolenic (18:3, n-3)</td>
<td>3.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Behenic (22:0)</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Arachidononic (20:4, n-6)</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Eicosapentaenoic (20:5, n-3)</td>
<td>0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Nervonic (24:1, n-9)</td>
<td>0.6</td>
<td>0.09</td>
</tr>
<tr>
<td>Docosahexaenoic (22:6, n-3)</td>
<td>1.3</td>
<td>trace amount</td>
</tr>
</tbody>
</table>

Fatty acid composition of the experimental diets was determined by means of gas-liquid chromatography. All values are expressed as mol %.
antibodies specific to each PPAR isofom (Santa Cruz Biotechnology). After incubation with the secondary alkaline phosphatase-conjugated antibody (Sigma) protein bands were scanned and quantified using a Gel Doc EQ system (Bio-Rad).

Statistical analysis

All data are presented as means ± SD. Statistical comparisons were made by using two-way ANOVA followed by Newman-Keuls post-hoc test. If variances were heterogeneous among groups (Levene's test, P<0.05), Welch ANOVA and Dunnett's T3 post-hoc test were used instead. P<0.05 was considered statistically significant. One, two, or three symbols indicate a significant difference at the P<0.05, P<0.01, or P<0.001 levels, respectively.

RESULTS

General features of the experimental animals

High-fat feeding resulted in higher weight gain compared to the standard chow fed group, however, the difference did not reach statistical significance (Table 2). Administration of WY-14643 did not affect weight gain in either diet group. High-fat diet elevated plasma FFA and decreased plasma TAG concentration (Table 2). Administration of PPARα agonist to the animals fed on the standard chow reduced plasma FFA and TAG concentration by 27 and 15%, respectively. On the contrary, in the high-fat fed rats plasma FFA and TAG concentration was increased after WY-14643 treatment by 23 and 45%, respectively.

Table 2. Weight gain and plasma lipids in the experimental groups.

<table>
<thead>
<tr>
<th>WY-14643</th>
<th>Standard chow</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>70.4±23.4</td>
<td>75.8±21.6</td>
</tr>
<tr>
<td>Plasma FFA (nmol/ml)</td>
<td>256±46</td>
<td>188±33*</td>
</tr>
<tr>
<td>Plasma TAG (nmol/ml)</td>
<td>1438±162</td>
<td>1219±65***</td>
</tr>
</tbody>
</table>

Values are means ± SD (n=10). FFA – free fatty acids, TAG – triacylglycerols. * significant difference vs. the untreated group fed on the standard chow, † significant difference vs. the untreated group fed on the high-fat diet.

Fig. 1. Effects of WY-14643 (WY) and high-fat diet on the myocardial content of fatty acids in the fractions of diacylglycerols, triacylglycerols, phospholipids and free fatty acids. Rats were fed either a standard chow (white bars) or a high-fat diet (grey bars) for three weeks and were treated or not with WY-14643 in a dose of 3 mg/kg/d. Values are means ± SD, n=10. * significant difference vs. the untreated group fed on the standard chow, † significant difference vs. the untreated group fed on the high-fat diet, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.
Content of myocardial lipids

Administration of WY-14643 induced opposite effects on the total intramyocardial FFA content in the rats fed standard (43% reduction) and high-fat diet (46% increase) (Fig. 1). These changes were, however, limited to SAT and MUFA as the level of PUFA in this fraction was not affected by PPARα agonist in either group. High-fat diet alone decreased the total content of myocardial FFA by 29% which was a result of reduced level of SAT and MUFA in this fraction. However, the change reached statistical significance only in the case of MUFA.

WY-14643-induced effects on the total DAG-fatty acid content in the heart also depended on dietary fat intake (Fig. 1). In the group fed standard chow PPARα agonist decreased DAG level by 34%, whereas in the high-fat fed rats the compound induced a 26% increase in the myocardial DAG content. These changes were a result of alterations in the level of saturated and monounsaturated DAG species. On the other hand, high-fat diet alone affected neither total DAG-fatty acid content nor the level of individual groups of DAG-fatty acids.

PPARα agonist did not produce statistically significant changes in the total content of TAG-fatty acids in either group (Fig. 1). However, in the high-fat fed rats WY-14643 induced a trend towards increased TAG level resulting from elevated content of saturated TAG species. High-fat diet alone markedly decreased the level of saturated TAG-fatty acids (by 62%), whereas the content of PUFA in this fraction was simultaneously elevated 2.7-fold. The net effect of these changes was a 25% reduction in the total TAG content.

Administration of WY-14643 to the animals fed standard chow increased the content of all groups of PH-fatty acids which resulted in a 17% elevation in the total level of PH-fatty acids in the heart (Fig. 1). However, in the high-fat fed group the compound had no effect on either total PH-fatty acid content or the level of individual groups of PH-fatty acids. High-fat diet alone increased the total content of PH-fatty acids by 10% which was a consequence of elevation in the level of SAT and PUFA in this fraction. It should be noted, however, that the content of PH-MUFA was simultaneously decreased by 26%.

Fatty acid composition of myocardial PH and plasma FFA

WY-14643 exerted similar effects on myocardial PH fatty acid composition in both diet groups (Table 3). In general, these changes were reflected by increased percentage of MUFA (with the exception of palmitoleic acid) and elevated n6/n3-PUFA ratio. The latter effect was a result of simultaneous decrease in the percentage of n3-PUFA (with the exception of α-linolenic acid) and increase in the total percentage of n6-PUFA. However, it should be noted that in the group fed standard chow the increase in the total percentage of n6-PUFA was a consequence of elevated percentage of arachidonic acid, whereas in the high-fat fed rats it resulted from increased percentage of linoleic acid.

High-fat diet alone induced profound changes in the fatty acid composition of myocardial PH. Among all investigated fatty acids only the percentage of docosahexaenoic acid remained stable. In general, high-fat feeding decreased the percentage of all MUFA and slightly elevated the total percentage of n6-PUFA. In the latter group of PH-fatty acids linoleic acid was to a large extent replaced by arachidonic acid. Among n3-PUFA the percentage of α-linolenic and eicosapentaenoic acid was markedly decreased by high-fat diet whereas the percentage of docosahexaenoic acid remained stable.

In general, WY-14643 exerted moderate effect on plasma FFA. It should be noted, however, that the changes observed in the standard chow fed rats were more pronounced (Table 4). The common effect of PPARα agonist in both diet groups was the

Table 3. Effect of WY-14643 on the fatty acid composition of myocardial phospholipids.

<table>
<thead>
<tr>
<th> </th>
<th>Standard chow</th>
<th> </th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WY-14643</strong></td>
<td> </td>
<td> </td>
<td> </td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>0.178±0.031</td>
<td>0.136±0.013</td>
<td>0.08±0.01***</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>15.2±0.84</td>
<td>15.4±0.95</td>
<td>9.12±0.62***</td>
</tr>
<tr>
<td>Palmitoleic (16:1, n-7)</td>
<td>0.909±0.077</td>
<td>0.716±0.1**</td>
<td>0.11±0.02***</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>22.3±1.03</td>
<td>21.5±0.55</td>
<td>29±0.37***</td>
</tr>
<tr>
<td>Oleic (18:1, n-9)</td>
<td>4.99±0.47</td>
<td>6.11±0.45***</td>
<td>3.84±0.24***</td>
</tr>
<tr>
<td>Linoleic (18:2, n-6)</td>
<td>34.2±2.54</td>
<td>31.5±2.52*</td>
<td>28±1.29***</td>
</tr>
<tr>
<td>Arachidic (20:0)</td>
<td>0.328±0.034</td>
<td>0.291±0.027</td>
<td>0.22±0.01***</td>
</tr>
<tr>
<td>α-Linolenic (18:3, n-3)</td>
<td>0.098±0.01</td>
<td>0.109±0.013</td>
<td>0.025±0.004***</td>
</tr>
<tr>
<td>Behenic (22:0)</td>
<td>0.297±0.011</td>
<td>0.17±0.02***</td>
<td>0.42±0.02***</td>
</tr>
<tr>
<td>Arachidonic (20:4, n-6)</td>
<td>13.2±0.78</td>
<td>17.7±1.69***</td>
<td>20.4±1.02***</td>
</tr>
<tr>
<td>Eicosapentaenoic (20:5, n-3)</td>
<td>0.217±0.029</td>
<td>0.15±0.04***</td>
<td>0.04±0.01***</td>
</tr>
<tr>
<td>Nervonic (24:1, n-9)</td>
<td>0.158±0.016</td>
<td>0.185±0.022*</td>
<td>0.1±0.02***</td>
</tr>
<tr>
<td>Docosahexaenoic (22:6, n-3)</td>
<td>7.77±1.4</td>
<td>5.86±0.9***</td>
<td>8.04±0.78</td>
</tr>
<tr>
<td>Lignoceric (24:0)</td>
<td>0.169±0.009</td>
<td>0.197±0.039*</td>
<td>0.212±0.01***</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>38.4±0.94</td>
<td>37.6±1</td>
<td>39.1±0.48</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>6.06±0.5</td>
<td>7.01±0.53***</td>
<td>4.05±0.25***</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>55.5±0.52</td>
<td>55.4±0.94</td>
<td>56.9±0.46***</td>
</tr>
<tr>
<td>Σ n3-PUFA</td>
<td>8.09±1.41</td>
<td>6.12±0.89***</td>
<td>8.1±0.79</td>
</tr>
<tr>
<td>Σ n6-PUFA</td>
<td>47.4±1.8</td>
<td>49.2±1.1*</td>
<td>48±1*</td>
</tr>
<tr>
<td>n6/n3-PUFA</td>
<td>6.02±1.09</td>
<td>8.22±1.43***</td>
<td>6.08±0.74</td>
</tr>
</tbody>
</table>

Values are mol % ± SD (n=10). SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, n.d. – not detected. * significant difference vs. the untreated group fed on the standard chow, † significant difference vs. the untreated group fed on the high-fat diet.
increase in the percentage of oleic acid. The compound did not induce statistically significant changes in the percentage of any group of free fatty acids in the high-fat fed animals. However, in the standard chow fed rats a profound reduction in the total percentage of n3-PUFA resulting from decreased percentage of α-linolenic and docosahexaenoic acid was observed. There was also a strong trend towards increased n6/n3-PUFA ratio in this group which, however, did not reach statistical significance (P=0.28).

High-fat diet alone induced marked changes in the composition of plasma FFA. In general, saturated, monounsaturated and n-3 polyunsaturated fatty acids were to a large extent replaced by n6-PUFA, the percentage of which has doubled. In consequence the n6/n3-PUFA ratio increased over 4-fold.

WY-14643 did not affect myocardial 2-deoxyglucose uptake in either group, whereas, high-fat diet alone decreased it by 68% (Fig. 2a). Administration of PPARα agonist elevated glycogen content in the heart by 78 and 43% in rats fed on the standard and the high-fat diet, respectively (Fig. 2b). In the standard chow fed rats WY-14643 decreased the index of cardiac palmitate oxidation (Rpox) by 37%, whereas in the high-fat fed animals it was not affected by the drug (Fig. 2c).

Myocardial expression of PPARα and δ

High-fat diet alone moderately increased the myocardial content of PPARα and δ protein (Fig. 3). WY-14643 administration did not affect the content of PPARα protein in the heart of the standard chow fed animals whereas in the high-fat fed rats the drug induced a modest decrease in the receptor protein expression. On the other hand, the myocardial level of PPARδ was decreased by WY-14643 treatment in both diet groups (by 17 and 29% in rats fed on the standard and high-fat diet, respectively).

DISCUSSION

To the best of our knowledge this is the first report describing effects of PPARα activator on the content and fatty acid composition of all major glycerolipid classes in the heart. We showed that effect of WY-14643 on the level of myocardial lipids to a large extent depended on dietary fat intake. Administration of the drug to the rats fed on the standard chow markedly reduced FFA and DAG content in the heart, whereas in the high-fat fed animals the opposite effect was observed. Similar findings were reported in our previous paper where WY-14643 was shown to differently affect sphingolipid metabolism in the heart of rats fed standard chow and high-fat diet (3). In general, the above mentioned changes in the myocardial content of FFA and DAG reflected effects of the drug on plasma lipid availability. This hypothesis is also supported by the effect of the PPARα agonist on cardiac
Palmitate oxidation. In the standard chow fed animals WY-14643 paradoxically decreased the content of acid soluble metabolites of radiolabeled palmitate in the heart which was likely a consequence of the lipid lowering action of the drug. Therefore, increased fatty acid flux through the oxidative pathway could not contribute to the observed decrease in myocardial FFA and DAG. On the other hand, in the high-fat fed rats WY-14643 did not affect palmitate oxidation in the heart which further indicates that the increase in the content of myocardial lipids in this group was a consequence of elevated availability of blood-borne fatty acids. Unfortunately, there is no literature data on effects of PPARα agonists on the myocardial content of FFA and DAG. In our experiment PPARα activation did not affect TAG level in the heart of rats fed on either diet which is in line with other studies (2, 24, 32).

It was shown that high-fat feeding can reduce spontaneous physical activity in rats (26). Since we do not have data on the level of physical activity in different experimental groups, we cannot exclude the possibility that this factor might have had an impact on parameters of lipid metabolism measured in our study. It is therefore plausible that diet-dependent differences in the effect of WY-14643 on myocardial lipid metabolism were a consequence of PPARα activation on the background of not only distinct fat intake, but also distinct level of physical activity.

As already mentioned in the introduction numerous experiments conducted on cultured cardiac myocytes revealed that PPARα activation induces expression of a wide array of genes involved in fatty acid catabolic pathways. However, most in vivo studies have shown little or no effect of PPARα agonists on myocardial expression of genes regulated by this receptor (1, 9, 20). Moreover, synthetic PPARα activators were paradoxically found to decrease cardiac fatty acid oxidation rate in murine models of diabetes and diet-induced obesity (1, 2) which is in line with the results of our study. This discrepancy between in vitro and in vivo studies is generally thought to be a consequence of the fact that in vivo PPARα transcriptional activity is limited by receptor expression rather than ligand availability. Indeed, in the study by Finck et al. (13) it was shown that WY-14643 upregulates PPARα-dependent genes in the heart of mice with cardiac-restricted overexpression of PPARα but not in their wild-type littermates. Taking together, it is very likely that effects of WY-14643 on the content of myocardial lipids observed in our study were indirect and secondary to changes in systemic lipid metabolism.
An interesting finding of our study is that WY-14643 administration induced a decrease in the myocardial expression of PPARα in both diet groups. This effect could rather not be secondary to changes in systemic lipid metabolism since PPARα agonist induced opposite effects on plasma lipid concentration in each diet group. Experiments on cultured rodent cardiomyocytes revealed that PPARα and PPARδ regulate similar set of genes involved in fatty acid catabolism (43). It was also shown that cardiomyocyte-restricted PPARδ knockout mice exhibit markedly lower myocardial fatty acid oxidation rate (7). It is therefore tempting to speculate that in the heart selective PPARα stimulation may negatively affect PPARδ expression and in consequence also its transcriptional activity. This mechanism might contribute to the lack of increase in the myocardial palmitate oxidation rate following WY-14643 administration.

In the present experiment PPARα activator did not affect basal myocardial glucose uptake which is in line with the study by Finck et al. (13) showing no effect of WY-14643 on GLUT1 expression in the murine heart. Moreover, Ye et al. (35) reported that WY-14643 did not modify myocardial glucose uptake during euglycemic-hyperinsulinemic clamp in high-fat fed rats. A new finding of our study is that PPARα agonist markedly increased glycogen content in the heart. Mandard et al. (19) reported that murine glycogen synthase 2 (Gys-2) gene contains active PPAR response elements and that PPARα is a transcriptional regulator of Gys-2 expression in liver and white adipose tissue. They also showed that WY-14643 markedly increases Gys-2 expression in isolated rat hepatocytes. Therefore, it is plausible that PPARα agonist-induced elevation in the myocardial glycogen content observed in our study was a consequence of its increased synthesis resulting from higher glycogen synthase expression. However, it is not known whether the muscle isoform of this enzyme is also under transcriptional control of PPARα.

WY-14643 exerted profound influence on the fatty acid composition of myocardial phospholipids. These changes were to a large extent independent from dietary fat intake and in general, included increased percentage of MUFA and replacement of n3-PUFA by n6-PUFA. Our results are in contrast to an earlier study by Tian et al. (30) that found markedly decreased n6/n3-PUFA ratio in myocardial lipids in rats treated with clofibrate. It should be noted, however, that they determined fatty acid composition of total lipid extracts, whereas in our study it was analyzed in the fraction of phospholipids isolated by means of TLC. This likely contributed to the observed discrepancy.

WY-14643-induced elevation in the percentage of MUFA in the myocardial phospholipids was most likely a consequence of increased availability of oleic acid, since administration of the drug increased the percentage of this fatty acid in plasma FFA in both diet groups. Increased percentage of oleic acid in plasma lipids after treatment with a PPARα agonist was observed also by Christiansen et al. (8). This action of PPARα activators is thought to be a result of increased expression of liver stearoyl-CoA desaturase (23). Although WY-14643 induced similar changes in phospholipid PUFA composition in both diet groups the mechanism of its action seems to be different in each group. In the standard chow fed rats alterations in the myocardial phospholipid PUFA composition generally reflected those observed in plasma FFA which indicates that the effect of PPARα agonist was indirect and secondary to changes in systemic PUFA metabolism. Indeed it was shown that PPARα activation stimulates Δα- and Δα-Δα-desaturase expression as well as n6-PUFA synthesis in the rat liver (16, 21). On the other hand, in the high-fat fed animals WY-14643 did not affect either n6/n3-PUFA ratio or the percentage of any of plasma free PUFA which suggests direct action of the drug on PUFA metabolism in the heart.

There is abundant evidence that n3-PUFA are beneficial to the cardiovascular system. Clinical trials showed that intake of fish oil rich in eicosapentaenoic and docosahexaenoic acid reduces the mortality due to myocardial infarction. Moreover, experimental studies on rodents revealed that enrichment of myocardial membranes with n3-PUFA has anti-arrhythmic effect, reduces ischemic damage of the heart and increases post-ischemic recovery (22). Therefore, decreased contribution of n3-PUFA in the myocardial phospholipids that was observed in our study after PPARα agonist administration should be considered as an unfavourable effect of the drug that might have detrimental effects on the heart. Our results indicate that the aforementioned effect of WY-14643 is not transient, since it was still present after 14 days of drug administration. However, additional experiments using more chronic treatment are necessary to fully address this issue.

In summary, we found that WY-14643 induced reciprocal effects on the myocardial content of FFA and DAG in rats fed on the standard and high-fat diet. These changes followed alterations in plasma lipid concentration which along with the lack of increase in palmitate oxidation rate in the heart implies indirect action of PPARα agonist on cardiac lipid metabolism. Administration of WY-14643 had profound influence on fatty acid composition of myocardial phospholipids with the most notable effect being increased n6/n3-PUFA ratio. This action of PPARα activator might be detrimental to the heart since n3-PUFA possess cardioprotective and antiarrhythmic properties.

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