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M. BARANOWSKI, A. BŁACHNIO, P. ZABIELSKI, J. GÓRSKI

PPARα AGONIST INDUCES THE ACCUMULATION OF CERAMIDE IN THE HEART OF RATS FED HIGH-FAT DIET

Department of Physiology, Medical University of Bialystok, Bialystok, Poland

It was shown that high-fat feeding of mice with cardiac-specific overexpression of peroxisome proliferator-activated receptor (PPAR) α but not wild type animals leads to the accumulation of ceramide (an important mediator of lipotoxicity) in the heart [Finck et al. 2003 Proc Natl Acad Sci USA]. To investigate the mechanism of this phenomenon we examined the effects of PPARα activation on ceramide metabolism in the myocardium. Male Wistar rats were fed either a standard chow or a high-fat diet. Each group was divided into two subgroups: control and treated with selective PPARα activator – WY-14643. In the rats fed on the standard diet WY-14643 did not affect the myocardial content of sphingomyelin and ceramide but reduced the content of sphinganine and sphingosine. It also inhibited the activity of neutral sphingomyelinase and increased the activity of acid sphingomyelinase, whereas the activity of ceramidases and serine palmitoyltransferase (SPT) remained stable. High-fat diet itself did not affect the content of the examined sphingolipids. However, it reduced the activity of sphingomyelinases and ceramidases having no effect on the activity of SPT. Administration of WY-14643 to this group significantly increased the content of myocardial free palmitate, ceramide, sphingomyelin and the activity of SPT. Our results demonstrated that PPARα activation modulates myocardial ceramide metabolism and leads to the accumulation of ceramide in the heart of the high-fat fed rats due to its increased synthesis de novo.

Keywords: *lipid signaling, ceramide, PPAR, high-fat diet, serine palmitoyltransferase*

INTRODUCTION

A considerable portion of the muscle total lipids consists of sphingolipids. These lipids have been shown to have not only a structural function in biological membranes but also to play a significant role in the transmembrane

signal transduction and as a result in regulation of various cellular processes such as proliferation, differentiation, apoptosis and inflammation in different tissues (1). The first step of so-called sphingomyelin signaling pathway is hydrolysis of sphingomyelin (SM) by the action of the enzyme sphingomyelinase to yield second messenger – ceramide (CER). Ceramide is also synthesized de novo in Golgi apparatus (*Fig. 1*). The first and rate-limiting step in the latter pathway is condensation of serine and palmitoyl-CoA to form 3-ketosphinganine. This reaction is catalyzed by the enzyme serine

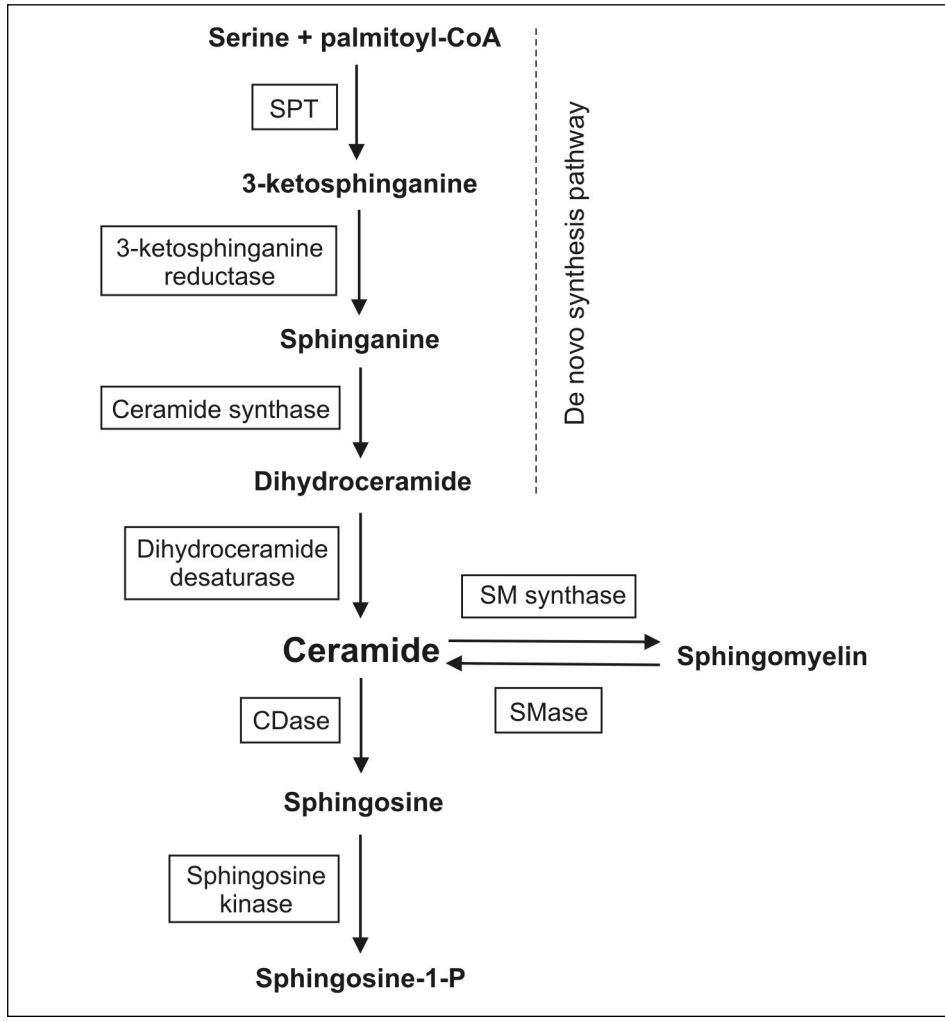

Fig. 1. Overview of ceramide metabolism. SPT – serine palmitoyltransferase, SM – sphingomyelin, SMase – sphingomyelinase, CDase – ceramidase.

palmitoyltransferase (SPT). CER is deacylated by the enzyme ceramidase. Sphingosine, the product of ceramide deacylation, can be further phosphorylated to form sphingosine-1-phosphate (S1P). Both sphingosine and S1P are bioactive sphingolipids (1). All the mediators and key enzymes of this pathway such as sphingomyelinase, ceramidase, SPT and sphingosine kinase were shown to be present in the myocardium (2-7). Although intensively studied, the regulation of the functioning of the sphingomyelin signaling pathway in the heart is not clear. It was shown that hypoxia-reoxygenation rapidly activates neutral Mg^{2+}-dependent sphingomyelinase (N-SMase) in rat cardiomyocytes which results in the accumulation of ceramide (8). However, Zhang et al. (4) reported a reduction in the activity of cardiac N-SMase, acid sphingomyelinase (A-SMase) and ceramidase with a concomitant increase in the content of CER in a rat model of ischemia-reperfusion injury. It was also shown that increased availability of extracellular palmitate leads to accumulation of ceramide in cardiomyocytes, which is likely a result of its augmented synthesis de novo (9). Sphingolipid metabolism in the heart is also affected by exercise, which decreases the activity of both N- and A-SMase and as a result it reduces the content of CER (10).

Peroxisome proliterator-activated receptors (PPARs) are ligand-activated transcription factors of the nuclear hormone receptor superfamily. Three distinct PPAR isoforms termed α, δ and γ have been described, of which all are expressed in the heart. PPARα is a well known transcriptional regulator of lipid metabolism in cardiomyocytes. Activation of this receptor induces the expression of a number of genes encoding proteins involved in transmembrane transport and mitochondrial β-oxidation of fatty acids. In consequence, PPARα activation increases the uptake and oxidation of fatty acids in the heart (11). However, a role of this receptor in the regulation of metabolism of other lipids is poorly understood. There are some data in the literature indicating that PPARα agonists modulate cellular sphingolipid metabolism. Chocian et al. (12) found that administration of bezafibrate, a PPARα activator, increases the level of ceramide in rat liver nuclei in vivo. PPARα agonists were also shown to reduce myocardial infarct size in a rat model of ischemia-reperfusion injury (13), a condition in which sphingolipid signaling pathway is activated in the heart (3, 4, 14). Recently Finck et al. (15) demonstrated that PPARα-mediated lipotoxicity plays an important role in the pathogenesis of diabetic cardiomyopathy. They found that high-fat feeding of mice with cardiac-specific overexpression of PPARα leads to the accumulation of triacylglycerols and ceramide [an important mediator of lipotoxicity (16)] in the myocardium. Such effect was not observed in the wild type animals which suggests that PPARα may be involved in the regulation of myocardial CER metabolism. To investigate the mechanism of this phenomenon we examined the effects of PPARα activation on the level of key intermediates and the activity of principal enzymes of ceramide metabolism in the rat heart.

MATERIAL 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 (200-240 g) were housed in separate cages under controlled conditions (21 °C ± 2, 12 h light/12 h dark cycle) with unlimited access to water. The animals were randomly divided into two groups: 1) fed ad libitum on the standard laboratory rat chow (Agropol, Motycz, Poland) containing 2.8 % of fat by weight (n=20), 2) fed for three weeks on the high-fat diet containing 33.9 % of fat (sunflower oil) by weight (n=20), prepared as described by Pascoe and Storlien (17). The diet was administrated daily in the amount providing a number of calories corresponding to the previously determined average energy intake of rats fed on a standard chow (82 kcal per animal per day). The fatty acid composition of the experimental diets is presented in *Table 1*. Each group was further divided into two subgroups: a) control (n=10) and b) treated daily for two weeks with a selective PPARα agonist, WY-14643 (Cayman Chemicals) in a dose of 3 mg/kg of body weight starting from the second week of the experiment (n=10). The drug was suspended in 0.5 % methylcellulose and administrated by an oral gavage.

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

Sphingomyelin and ceramide fatty acid content

The samples were pulverized in an aluminum mortar precooled in liquid nitrogen. The powder was then transferred to a tube containing methanol and 0.01% butylated hydroxytoluene (Sigma) as an antioxidant. Lipids were extracted by the method of Folch. Next, ceramide and sphingomyelin were isolated with thin-layer chromatography (TLC) using the methods described by Yano et al. (18) and Mahadevappa et al. (19) respectively. Further analysis was performed as previously

Table 1. Fatty acid composition of the experimental diets.

	Standard chow	**High-fat diet**
Myristic (14:0)	2	0,3
Palmitic (16:0)	21,8	8,1
Palmitoleic (16:1)	1,4	0,2
Stearic (18:0)	12,8	4,4
Oleic (18:1)	20	28,1
Linoleic (18:2)	35,5	57,6
Arachidic (20:0)	0,3	0,2
Linolenic (18:3)	3,4	0,1
Behenic (22:0)	0,2	0,8
Arachidonic (20:4)	0,1	0,03
Eicosapentaenoic (20:5)	0,6	0,05
Nervonic (24:1)	0,6	0,09
Docosahexaenoic (22:6)	1,3	trace amount

Fatty acid composition of the experimental diets was determined by means of gas-liquid chromatography. All values are expressed as mol %.

described in detail (20). Briefly, the gel bands corresponding to the standards were scrapped off the plates and transferred into screw-cap tubes containing pentadecanoic acid (Sigma) as an internal standard. Ceramide and sphingomyelin fatty acids were then transmethylated in the presence of 14 % boron trifluoride (Sigma) in methanol at 100 °C for 90 min. Fatty acid methyl esters were analyzed by means of gas-liquid chromatography. A Hewlett-Packard 5890 Series II system equipped with a double flame ionization detector and Agilent CP-Sil 88 capillary column (100 m, 0.25 mm i.d.) were used.

The content of myocardial free palmitate

Lipids were extracted as described above and the fraction of free fatty acids (FFA) was isolated by means of TLC according to Roemen and van der Vusse (21). The gel bands corresponding to the FFA standard were scrapped off the plates and transferred into fresh tubes. FFA were then transmethylated and the content of palmitate methyl ester was determined by means of gas-liquid chromatography as previously described in detail (22).

The content of sphingosine, sphinganine and sphingosine-1-phosphate

The content of sphingosine, sphinganine and S1P was measured simultaneously by the method of Min et al. (23). Briefly, tissues were homogenized in a solution composed of 25 mM HCl and 1 M NaCl. Acidified methanol and internal standards (C_{17}-sphingosine and C_{17}-S1P, Avanti Polar Lipids) were added and the samples were ultrasonicated in ice-cold water for 1 min. Lipids were then extracted by the addition of chloroform, 1 M NaCl and 3 N NaOH. The alkaline aqueous phase containing S1P was transferred to a fresh tube. The residual S1P in the $CHCl_3$ phase was re-extracted twice with methanol /1 M NaCl (1:1, v/v) solution and then all the aqueous fractions were combined. The amount of S1P was determined indirectly after dephosphorylation to sphingosine with the use of alkaline phosphatase (bovine intestinal mucosa, Fluka). To improve the extraction yield of released sphingosine some chloroform was carefully placed at the bottom of the reaction tubes. The $CHCl_3$ fractions containing free sphingosine and sphinganine or sphingosine liberated from S1P were washed three times with alkaline water (pH adjusted to 10.0 with NH_4OH) and then evaporated under a nitrogen stream. The dried lipid residues were redissolved in ethanol, converted to their o-phthalaldehyde derivatives and analyzed on a HPLC system (ProStar, Varian Inc.) equipped with a fluorescence detector and C18 reversed-phase column (Varian Inc. OmniSpher 5, 4.6 mm i.d. × 150 mm). The isocratic eluent composition of acetonitrile (Merck):water (9:1, v/v) and a flow rate of 1 ml/min were used.

The activity of sphingomyelinases

The activity of N- and A-SMase was determined as reported by Liu and Hannun (24). Briefly, the myocardial homogenates were centrifuged at 1000 × g for 10 min and 50 µl of the supernatant was used for further analysis. The activity of both sphingomyelinases was measured using radiolabeled substrate, [N-methyl-^{14}C]-sphingomyelin (Perkin-Elmer Life Sciences). For N-SMase the reaction mixture contained 100 nmol of sphingomyelin (1154 dpm/nmol) in 100 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.1 % Triton X-100 and 5 mM dithiothreitol in a final volume of 0.2 ml. In the case of A-SMase, the assay mixture contained 100 nmol of sphingomyelin (1154 dpm/nmol) in 100 mM sodium acetate (pH 5.0), 0.1 % Triton X-100 and 0.1 mM EDTA. After incubation at 37 °C for 1 h the reaction was stopped by adding 1.5 ml of chloroform:methanol (2:1 v/v), followed by addition of 0.2 ml of water. A portion of the aqueous phase was transferred to scintillation vials and counted in a liquid scintillation counter for the radioactivity of the reaction product, ^{14}C-choline phosphate.

The activity of ceramidases

The activity of alkaline (Al-CDase), neutral (N-CDase) and acid (Ac-CDase) ceramidase was determined by the method of Nikolova-Karakashian and Merrill (25). The activity of the enzymes was measured using radiolabeled substrate, [N-palmitoyl-1-^{14}C]-sphingosine (Moravek Biochemicals). The tissue homogenates were centrifuged at 1000 × g for 10 min and 50 µl of the supernatant was used for the analysis. The reaction was started by the addition of supernatant to the tubes containing 20 µl of substrate mixture (50 nmol of ceramide – 2353 dpm/nmol, 2.5 mg Triton X-100, 1 mg Tween 20, 0.4 mg sodium cholate) and 130 µl of a reaction buffer. The reaction buffer contained 125 mM sucrose, 0.01 mM EDTA and 125 mM sodium acetate (pH 4.5) or 100 mM Tris-HCl (pH 7.2) or 125 mM HEPES (pH 8.0) for Ac-CDaze, N-CDaze and Al-CDaze activity assay respectively. After incubation at 37 °C for 1 h the reaction was stopped by adding 2 ml of basic Doyle's solution (isopropanol:heptane:1 N NaOH, 40:10:1, v/v/v), 1.8 ml of heptane and 1.6 ml of water. Samples were then centrifuged and the upper phase was discarded. The lower phase was washed twice with 1.6 ml of heptane and then 1 ml of 1 N H_2SO_4 and 2.4 ml of heptane were added. After centrifugation, 1 ml aliquots from the upper phase were transferred to scintillation vials and analyzed for the radioactivity of the reaction product, ^{14}C-palmitate.

The activity of serine palmitoyltransferase

The activity of SPT was determined as reported by Merrill (26) using radiolabeled substrate, [^{3}H]-L-serine (Moravek Biochemicals). Briefly, myocardial homogenates were centrifuged for 10 min at 22000 × g and supernatant was recentrifuged at 150000 × g for 40 min to isolate microsomal fraction. The pellet was then resuspended in a buffer containing 5 mM HEPES (pH 7.4), 5 mM EDTA, 5 mM DTT and 20 % (w/v) glycerol. The reaction was started by addition of 20 µl of protein suspension to tubes prefilled with 180 µl of reaction buffer containing 100 mM HEPES (pH 8.3), 5 mM DTT, 2.5 mM EDTA (pH 7.0), 50 µM pyridoxal phosphate, 200 µM palmitoyl-CoA and 2 mM L-serine (44000 dpm/nmol). After incubation at 37 °C for 10 min the reaction was stopped by adding 0.2 ml of 0.5 N NH_4OH. The reaction product was extracted by the addition of 1.5 ml of chloroform:methanol (1:2, v/v). Samples were then partitioned with 1 ml of $CHCl_3$ and 2 ml of 0.5 N NH_4OH. The aqueous phase was discarded and the lower organic layer was washed three times with distilled water. A portion of the $CHCl_3$ phase was transferred to scintillation vials and analyzed for the radioactivity of the reaction product, ^{3}H-3-ketosphinganine.

Protein content

Protein content was measured with BCA protein assay kit (Sigma) according to the manufacturer's instructions. Bovine serum albumin (fatty acid free, Sigma) was used as a standard.

Statistical analysis

All data are presented as means ± SD. Statistical comparisons were made by using two-way analysis of variance followed by Newman-Keuls test. If variances were heterogeneous among groups, Dunnett's T3 test was used instead. $P < 0.05$ was considered statistically significant.

RESULTS

Sphingomyelin fatty acid content (Table 2)

There was no statistically significant difference in the total content of SM-fatty acids between the control groups fed on the standard and on the high-fat diet.

Table 2. Effects of WY-14643 and high-fat diet on the myocardial content of sphingomyelin-fatty acids.

	Standard diet		High-fat diet	
WY-14643	–	+	–	+
Myristic (14:0)	9.31±1.21	11.3±2.94	5.16±0.94 *	6.72±3.11
Palmitic (16:0)	161±22.4	149±18.7	121±8.35 *	213±30.5 #
Stearic (18:0)	113±8	102±19.5	101±5.03	181±36 #
Oleic (18:1)	7.18±1.68	9.67±1.76	9.53±1.24 *	22.4±3.61 #
Linoleic (18:2)	4.03±3.62	4.05±0.59	4.43±1.82	17.3±4.65 #
Arachidic (20:0)	106±9.99	99.9±13.3	76.4±8.36 *	122±24.3 #
Behenic (22:0)	99±8.63	90.5±10.7	226±11.2 *	344±74 #
Arachidonic (20:4)	5.8±0.86	7.22±1.35	4.26±0.74	9.91±2.78 #
Nervonic (24:1)	48±2.43	57.9±11	24.4±1.82 *	30.9±4.67 #
Docosahexaenoic (22:6)	2.8±0.38	3.06±0.7	4.14±0.63 *	4.99±1.05
Lignoceric (24:0)	86.7±5.48	96.3±26.5	103±3.95 *	146±26.4 #
SFA	**576±40.8**	**549±58.8**	**632±27.2 ***	**1012±182 #**
MUFA	**55.2±2.98**	**67.6±11.1 ***	**33.9±2.62 ***	**53.5±7.99 #**
PUFA	**12.6±3.46**	**14.3±1.78**	**12.8±2.56**	**32.2±6.89 #**
Total	**645±44.8**	**631±66.7**	**679±27.3**	**1098±190 #**

Values are nmol/g wet tissue ± SD (n=10). SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids. * $P<0.05$ vs. the control group fed standard diet, # $P<0.05$ vs. the control group fed high-fat diet.

However, the content of most of individual sphingomyelin-fatty acids was altered. As a result, the total content of sphingomyelin containing saturated fatty acids (SFA) increased whereas that containing monounsaturated fatty acids (MUFA) decreased. The content of SM-polyunsaturated fatty acids (PUFA) remained stable.

Administration of WY-14643 to the rats fed on the standard diet did not affect the total content of sphingomyelin fatty acids nor the content of individual sphingomyelins. In the high-fat fed rats the compound increased the total content of SM-fatty acids by 62 %. This was a result of elevation in the content of all individual sphingomyelins with the exception of those containing myristic and docosahexaenoic acid residues. The most profound change was seen in the case of SM containing linoleic acid residue, the content of which increased 3.9-fold.

Ceramide fatty acid content (Table 3)

The total content of CER in the control rats was not affected by the high-fat diet. However, the diet altered the content of most of individual ceramides. As a result, the total content of ceramide containing PUFA increased whereas that containing SFA and MUFA remained stable. Treatment with WY-14643 did not change neither the total content of ceramide fatty acids nor the content of individual ceramides in the rats fed on the standard diet. However, in the high-fat

Table 3. Effects of WY-14643 and high-fat diet on the myocardial content of ceramide-fatty acids.

	Standard diet		High-fat diet	
WY-14643	–	+	–	+
Myristic (14:0)	7.91±1.32	8.58±2.18	3.82±1.7 *	4.03±1.59
Palmitic (16:0)	24.5±5.37	27.2±6.5	23±8.42	37.4±10.1 #
Stearic (18:0)	25.1±5.54	22.8±4.42	20.4±5.71	39.8±11.5 #
Oleic (18:1)	5.49±1.29	7.55±3.59	9.23±2.64 *	19.2±6.69 #
Linoleic (18:2)	2.05±1.16	1.99±1.38	3.62±2.08	16.4±10.6 #
Arachidic (20:0)	5.16±0.84	5.29±0.48	4.57±0.46	7.1±1.16 #
Behenic (22:0)	6.42±0.87	6.73±1.2	15.8±1.96 *	25.8±3.34 #
Arachidonic (20:4)	2.52±0.88	1.75±0.79	4.35±0.6 *	12±7.09 #
Nervonic (24:1)	6.66±0.94	7.95±2.41	8.09±3.96	7.98±2.48
Docosahexaenoic (22:6)	2.89±0.62	2.58±0.51	4.3±1.26 *	6.74±2.48 #
Lignoceric (24:0)	12.3±0.76	14.6±8.67	15.8±1.03 *	23.8±3.23 #
SFA	**81.4±12.1**	**85.2±14.6**	**83.5±15.1**	**138±21.3 #**
MUFA	**12.1±1.86**	**15.5±4.29**	**17.3±5.98**	**27.4±8.74 #**
PUFA	**7.46±2.44**	**6.32±2.3**	**12.3±3.49 ***	**35.2±20 #**
Total	**101±14.9**	**107±19**	**113±23.9**	**201±44.9 #**

Values are nmol/g wet tissue ± SD (n=10). SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids. * P<0.05 vs. the control group fed standard diet, # P<0.05 vs. the control group fed high-fat diet.

fed rats, the total content of CER-fatty acids increased by 78 % after administration of the compound. This was accounted for by an elevation in the level of all individual ceramides with the exception of those containing myristic and nervonic acid residues. The most pronounced changes were observed in the case of CER containing linoleic and arachidonic acid residues, which increased 4.5- and 2.8-fold respectively.

The content of sphingosine, sphinganine and sphingosine-1-phosphate (Fig. 2)

The mean content of sphingosine, sphinganine and S1P in the heart of the control rats was 1.91±0.26, 0.53±0.11 and 0.42±0.08 nmol/g respectively and it was not affected by the high-fat diet. Administration of WY-14643 to the rats fed on the standard diet led to a small but significant reduction in the level of sphingosine and sphinganine (by 16 and 21 % respectively). In the high-fat fed rats WY-14643 did not affect the content of any compound.

The activity of sphingomyelinases (Fig. 3)

The high-fat diet markedly reduced the activity of both examined isoforms of sphingomyelinase. Treatment with WY-14643 reduced the activity of N-SMase and elevated the activity of A-SMase in the group fed on the standard diet and did not affect the activity of either enzyme in the high-fat fed group.

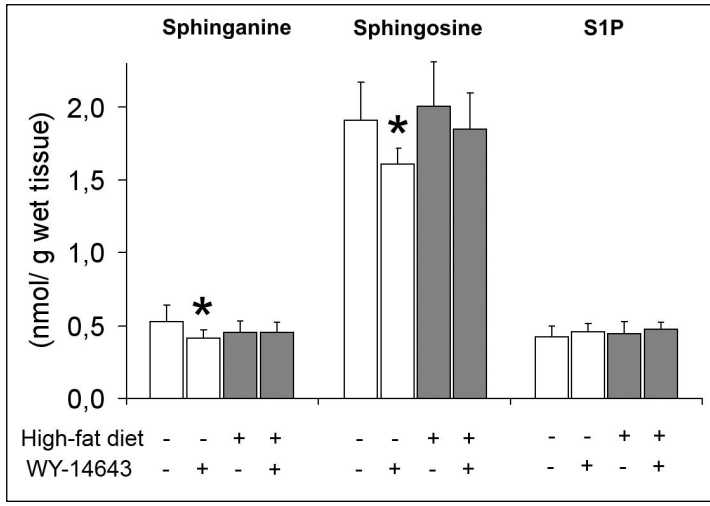

Fig. 2. The effect of WY-14643 and high-fat diet on the myocardial content of sphinganine, sphingosine and sphingosine-1-phosphate (S1P) (n=10). * P<0.05 vs. the control group fed standard diet.

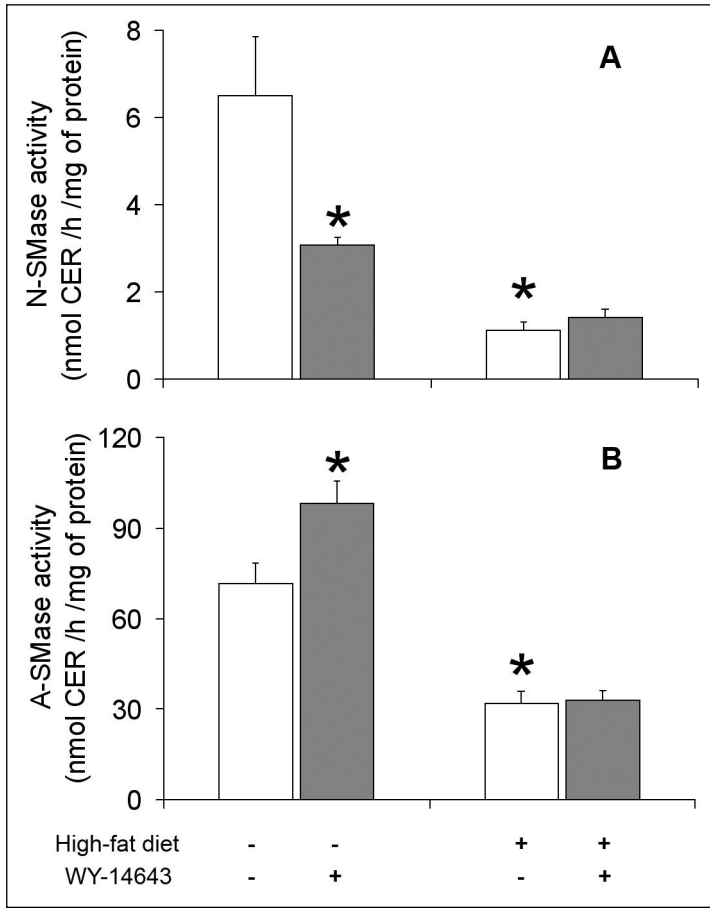

Fig. 3. Effects of WY-14643 and high-fat diet on the activity of (A) neutral Mg^{2+}-dependent sphingomyelinase (N-SMase) and (B) acid sphingomyelinase (A-SMase) in the heart (n=6). * P<0.05 vs. the control group fed standard diet (n=6). CER – ceramide.

The activity of ceramidases (Fig. 4)

The activity of the examined ceramidases was similar and amounted to 1.92±0.16, 1.97±0.34 and 1.62±0.28 nmol of sphingosine/h/mg of protein for Al-, N- and Ac-CDase respectively. The high fat diet itself reduced the activity of each enzyme. Treatment with WY-14643 did not affect the activity of either enzyme in both groups.

The activity of serine palmitoyltransferase (Fig. 5)

The activity of SPT in the heart of the control rats was 0.94±0.15 nmol of serine/h/mg of protein. Administration of WY-14643 to rats fed on the high-fat diet caused elevation in the activity of the enzyme by 23 %.

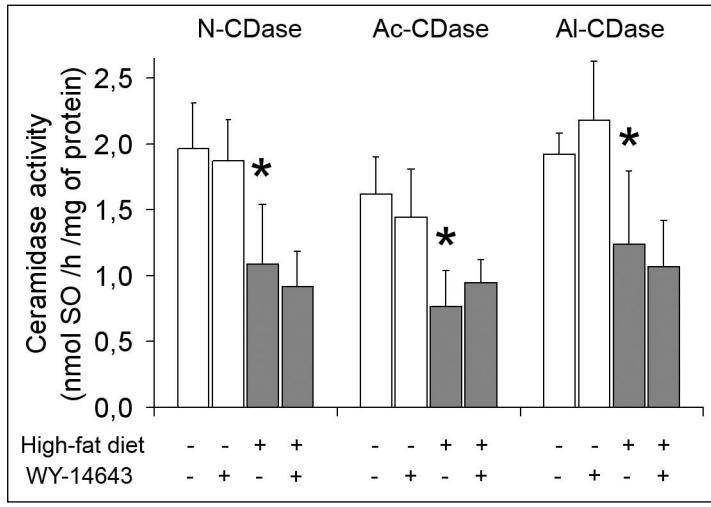

Fig. 4. Effects of WY-14643 and high-fat diet on the activity of alkaline (Al-CDase), neutral (N-CDase) and acid (Ac-CDase) ceramidase in the heart (n=6). * P<0.05 vs. the control group fed standard diet. SO – sphingosine.

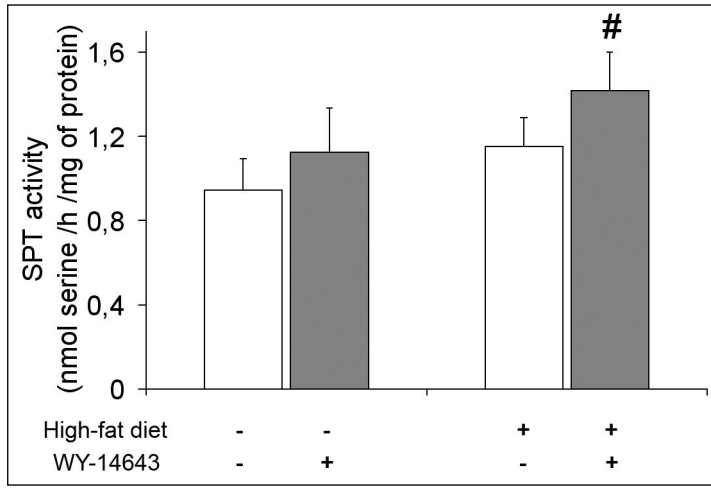

Fig. 5. Effects of WY-14643 and high-fat diet on the activity of serine palmitoyltransferase (SPT) in the heart (n=6). # P<0.05 vs. the control group fed high-fat diet.

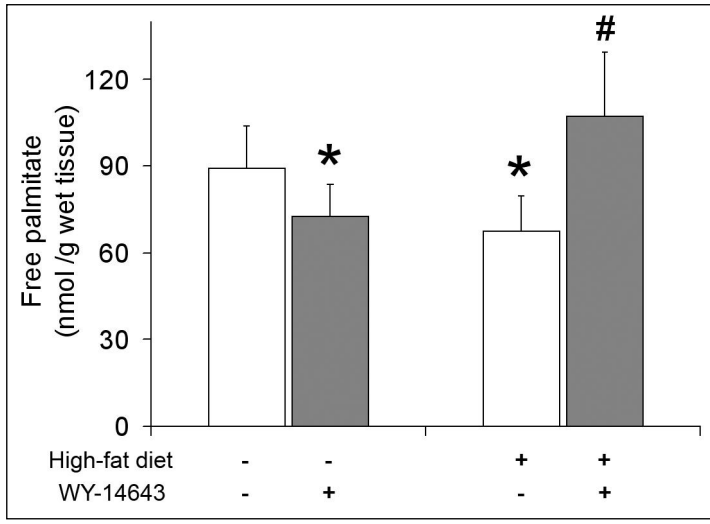

Figure 6. Effects of WY-14643 and high-fat diet on the myocardial content of free palmitate (n=10). * P<0.05 vs. the control group fed standard diet, # P<0.05 vs. the control group fed high-fat diet.

The content of myocardial free palmitate (Fig. 6)

Treatment with WY-14643 reduced the content of myocardial free palmitate in the group fed on the standard diet and increased it in the high-fat fed group.

DISCUSSION

A new finding of this study is that PPARα activation modulates sphingolipid metabolism in the heart and that this effect is largely dependent on the dietary fat intake. Stimulation of PPARα with WY-14643 in the rats fed on the standard diet did not affect the total content of either sphingomyelin or ceramide. Simultaneously, it exerted reciprocal effect on the activity of sphingomyelinases: the activity of N-SMase was reduced and the activity of A-SMase was elevated. The activity of A-SMase in the heart is considerably higher than the activity of N-SMase (4, 27) and it has been confirmed presently. It is, therefore, likely that the reduction of ceramide formation by the action of N-SMase was not matched by elevation of its formation by A-SMase and in consequence, an increase in the content of the compound could rather be expected, which was not the case in our study. No changes in the activity of ceramidases were found. It suggests that hydrolysis of ceramide was not affected by WY-14643. The latter is also supported by the fact that the content of sphingosine and S1P, the catabolites of ceramide, were stable. The activity of SPT was not affected by administration of WY-14643. However, the content of free palmitate in the heart and the content of sphinganine (a precursor on the de novo synthesis of CER) were reduced thus suggesting a reduction in the synthesis of ceramide due to decreased availability

of palmitate. Reduced synthesis of CER could counterbalance its increased formation by the action of A-SMase. These results strongly indicate that stimulation of PPARα may affect the rate of different routes of ceramide metabolism without affecting its content.

The high-fat diet did not affect the total content of either sphingomyelin or ceramide. As above, we also studied the activity of key enzymes regulating different routes of ceramide metabolism and we found that the diet did affect two of them. Stable activity of SPT and stable content of sphinganine would suggest that CER synthesis de novo remained unchanged after the diet. However, the diet markedly inhibited the activity of both sphingomyelinases, which indicates that formation of ceramide from sphingomyelin was reduced. Concomitantly, the activity of each isoform of ceramidase was significantly reduced thus indicating a reduction in the rate of CER hydrolysis. The latter obviously balanced the reduced rate of ceramide production due to reduced activity of sphingomyelinases.

There are very few data in the literature concerning the effects of increased dietary fat intake on the activity of the examined enzymes and the content of ceramide in different tissues. Yang at al. (28) reported a marked reduction in the activity of N- and A-SMase as well as N-CDase in the colonic mucosa of rats fed on a high-fat diet. On the other hand, Geelen and Beynen (29) observed an increase in the activity of N- and A-SMase in the liver of high-fat fed rats, which indicates that this response is tissue-dependent. Morgan et al. (30) did not find significant difference in the content of myocardial CER after feeding on a high-fat diet, rich in unsaturated fatty acids in rat infarct model of heart failure, which is in line with the results of the present study. Similar findings were reported in human and rat skeletal muscles after intravenous injection of lipid emulsions containing mostly unsaturated fatty acids (31, 32). On the other hand, Okere et al. (33) observed a reduction in the level of CER in the heart of rats fed on the high-fat diet comprised mainly of unsaturated fatty acids, while diet rich in SFA increased the amount of ceramide. However, they maintained the rats on the diet for 8 weeks, whereas we used a 3-week diet protocol which might contribute to the observed discrepancy.

Administration of WY-14643 to the high-fat fed rats, contrary to those fed on the standard diet, produced a marked elevation in the total content of CER and SM in the heart. Simultaneously, there were no changes in the activity of sphingomyelinases and ceramidases, which indicates that this effect was a result of augmented sphingolipid synthesis de novo. This is supported by the elevation in both the activity of SPT and the content of free palmitate in the myocardium. Hickson-Bick et al. (9) found that prolonged incubation of cultured neonatal rat cardiomyocytes in the presence of palmitate markedly elevates the content of ceramide. It was also shown that WY-14643 induces the expression and the activity of SPT in reconstructed human epidermis (34). However, the effect of PPARα agonists on the activity of this enzyme in the heart was not addressed previously. A mechanism of WY-14643 action on SPT activity remains obscure.

The results of the aforementioned study by Rivier et al. (34) suggest that it involves transcriptional regulation. However, it is not known whether PPARα directly stimulates the expression of SPT or it's an indirect effect of PPARα-induced changes in cellular lipid metabolism. Long-chain fatty acids were previously shown to increase the level of SPT mRNA in cultured rat pancreatic islets (35). Interestingly, in our study the activity of SPT was increased only in the group of animals, which showed a concomitant accumulation of free palmitate in the heart.

Ceramide has been shown to be a mediator of cardiomyocyte apoptosis induced by ischemia-reperfusion, tumor necrosis factor α (TNFα) and diabetes mellitus (2, 3, 36, 37). Moreover, Okere et al. (33) found a positive relationship between the myocardial content of CER and the number of apoptotic cardiomyocytes in rats on different diet regimens. It should be noted that in our study the magnitude of accumulation of myocardial CER after administration of WY-14643 to the rats fed on the high-fat diet was similar to that observed by others during ischemia-reperfusion injury (3, 8). It is therefore very likely that accumulation of ceramide in the heart of the high-fat fed rats treated with PPARα agonist could have detrimental effect on the myocardium.

We found that the effects of PPARα activation on sphingolipid metabolism in the heart are largely dependent on the dietary fat intake. Administration of WY-14643 to the rats fed on the standard diet did not affect the total content of SM and CER, whereas in the high-fat fed animals it produced an accumulation of the aforementioned lipids. This difference could be explained, at least in part, by the fact that the basal activity of sphingomyelinases and ceramidases was markedly lower in the high-fat fed group. Therefore, even a moderate increase in the rate of sphingolipid de novo synthesis, as that observed after WY-14643 treatment, could induce the accumulation of CER and in result also SM due to decreased rate of their degradation. Although increased availability of the substrate for sphingomyelin synthesis is a likely explanation for its accumulation, we cannot exclude the possibility that the activity of SM synthase in the heart was also induced by PPARα agonist in the high-fat fed rats. The fact that WY-14643 administration to the rats fed on the standard and on the high-fat diet produced opposite changes in the content of myocardial free palmitate indicates that also other effects of PPARα activation on cardiac lipid metabolism may depend on the dietary fat intake. It should be noted that fatty acids, either directly or indirectly, modulate the activity of a number of transcription factors including all PPAR isoforms (reviewed in 38). Therefore, PPARα activation in the state of increased availability of dietary fat may exert different effects due to altered transcription factors interplay. However, the elucidation of this phenomenon requires further investigation.

In summary, we found that in the rats fed on the standard diet WY-14643 did not affect the total content of CER, though it strongly inhibited the activity of N-SMase and elevated the activity of A-SMase. However, in the case of the high-fat

fed rats, the administration of PPARα agonist produced a marked increase in the total content of CER in the heart. It was likely a result of increased ceramide synthesis de novo, since there were no changes in the activity of sphingomyelinases and ceramidases, whereas the activity of SPT was elevated. We conclude that PPARα activation affects the metabolism of ceramide in the myocardium and that this effect is largely dependent on the dietary fat intake.

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Author's address: Jan Górski, Department of Physiology, Medical University of Bialystok, Mickiewicza 2c, 15-230 Bialystok, Poland. +48 85 748 55 85 (Phone), +48 85 748 55 86 (Fax); e-mail: gorski@amb.edu.pl