CHAPTER 3

HEART SPHINGOLIPIDS IN HEALTH AND DISEASE

Marcin Baranowski* and Jan Górski
Department of Physiology, Medical University of Białystok, Białystok, Poland
**Corresponding Author: Marcin Baranowski—Email: marcinb@umwb.edu.pl*

Abstract: In recent years, the role of sphingolipids in physiology and pathophysiology of the heart attracted much attention. Ceramide was found to be involved in the pathogenesis of cardiac dysfunction in animal models of ischemia/reperfusion injury, Type 2 diabetes and lipotoxic cardiomyopathy. On the other hand, another member of this lipid family, namely sphingosine-1-phosphate, has been shown to possess potent cardioprotective properties. This chapter provides a review of the role of ceramide and other bioactive sphingolipids in physiology and pathophysiology of the heart. We describe the role of PPARs and exercise in regulation of myocardial sphingolipid metabolism. We also summarize the present state of knowledge on the involvement of ceramide and sphingosine-1-phosphate in the development and prevention of ischemia/reperfusion injury of the heart. In the last section of this chapter we discuss the evidence for a role of ceramide in myocardial lipotoxicity.

INTRODUCTION

Sphingolipids were discovered over 120 years ago and for many decades were considered to serve only as structural components of biological membranes. Nowadays, many of them are known to be highly bioactive compounds that play a significant role in signal transduction and regulation of a host of cellular processes. Ceramide, the central molecule in sphingolipid structure and metabolism, is the best studied member of this family. It was first recognized as a second messenger in 1990 by Okazaki et al[1] who showed that ceramide mediates the effect of 1α,25-dihydroxyvitamin D_3 on HL-60 cell differentiation. Ceramide is also a precursor for other bioactive sphingolipids including

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ceramide-1-phosphate, sphingosine and sphingosine-1-phosphate (S1P) (Fig. 1). Intensive studies conducted over the next two decades revealed that sphingolipids regulate numerous cellular processes such as cell proliferation, differentiation and apoptosis as well as responses to cytokines and stress.[2] Ceramide has also emerged as a putative mediator of muscle insulin resistance and lipotoxicity in certain cell types, including cardiomyocytes.[3] In addition, the role of sphingolipids in the pathogenesis of cardiac dysfunction in ischemia/reperfusion injury, Type 2 diabetes and obesity has recently attracted much attention.

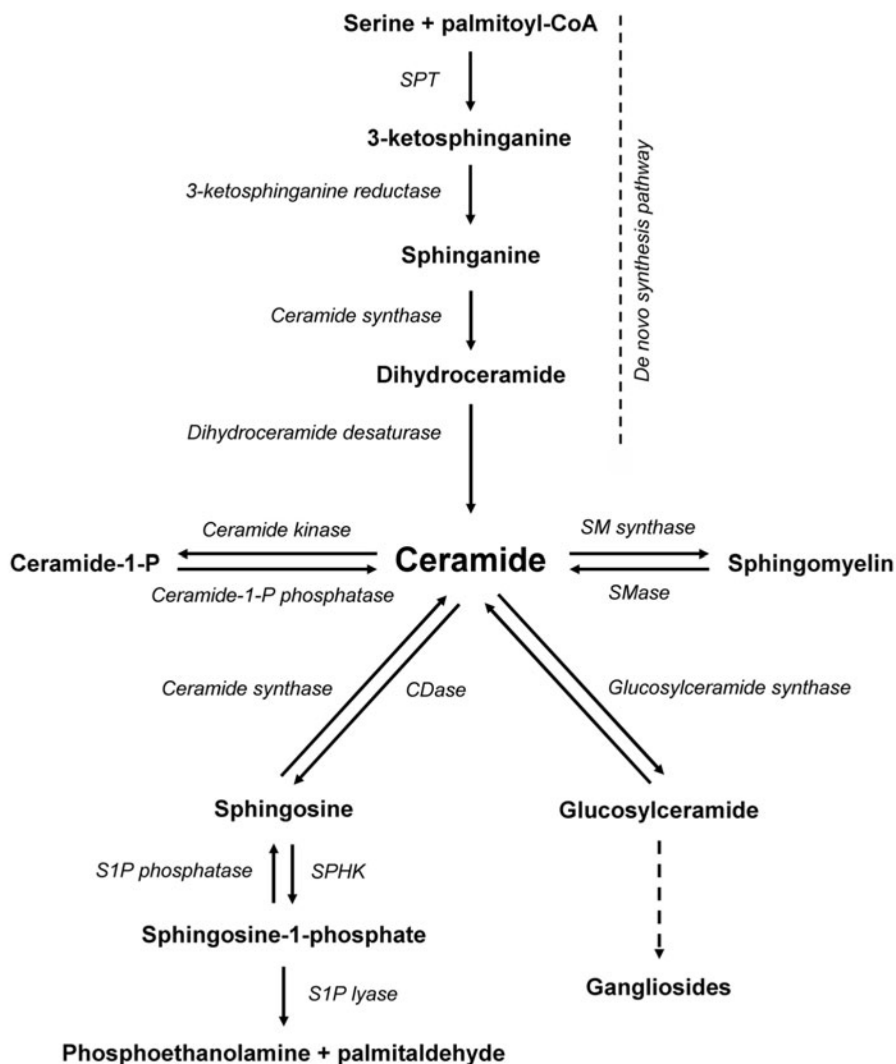

Figure 1. Schematic representation of ceramide metabolism. SPT-serine palmitoyltransferase, SM-sphingomyelin, SMase-sphingomyelinase, CDase-ceramidase, SPHK-sphingosine kinase, S1P-sphingosine-1-phosphate.

CHARACTERIZATION OF MYOCARDIAL SPHINGOLIPID METABOLISM

Both cardiac and skeletal muscle expresses different enzymes and receptors involved in sphingolipid metabolism and can, therefore, synthesize and respond to different bioactive sphingolipids. There are, however, considerable differences between myocardial and skeletal muscle sphingolipid metabolism. As shown on Table 1, the rat heart is characterized by approximately 2-fold higher content of sphingosine, S1P and sphingomyelin as compared to the soleus muscle, whereas the level of ceramide is virtually the same. The activity of most of the enzymes involved in sphingolipid metabolism is also much higher in the myocardium. We observed similar differences in the content of sphingoid bases also between human cardiac and skeletal muscle (see Table 1).

In addition, considerable species differences are found in myocardial sphingolipid metabolism. As compared to rat myocardium, human heart is characterized by similar level of ceramide, however, the content of sphingosine, sphinganine and S1P is substantially lower (Table 1). Another difference is relative contribution of individual ceramidase isoforms to total ceramidase activity. We showed that in the rat heart acid ceramidase (a-CDase) activity is much lower compared with activity of alkaline or neutral isoform of the enzyme.[4] However, in the human myocardium expression of a-CDase is markedly higher than alkaline/neutral-CDase, suggesting the dominance of the former isoform.[5] These results are in line with other reports showing high level of a-CDase mRNA in the

Table 1. Sphingolipid content and activity of enzymes related to ceramide metabolism in heart vs skeletal muscle

	Rat		Human	
	Soleus	Heart	Vastus Lateralis	Heart
Content of sphingolipids (pmol/mg)				
Sphingosine	1.0	2.2	0.33	1.3
Sphinganine	0.38	0.55	0.06	0.14
Sphingosine-1P	0.25	0.49	0.06	0.14
Ceramide	25	23	16	17
Sphingomyelin	316	645		
Enzyme activity (nmol of product/h/mg of protein)				
SPT	0.59	0.94		
al-CDase	1.5	6.0		
n-CDase	1.2	8.0		
a-CDase	not detected	2.7		
n-SMase	16.5	6.6		
a-SMase	23	125		

SPT-serine palmitoyltransferase, al-CDase-alkaline ceramidase, n-CDase-neutral ceramidase, a-CDase-acid ceramidase, n-SMase-neutral sphingomyelinase, a-SMase-acid sphingomyelinase. Created from data in M. Baranowski et al 2007 J Physiol Pharmacol, M. Baranowski et al 2010 J Lipid Res, Błachnio-Zabielska et al 2008 J Cell Biochem and from unpublished results of M. Baranowski.

human but not murine heart.[6,7] We found similar species differences also for expression of sphingosine kinase isoforms.[5] Our data indicate that sphingosine kinase (SPHK) 1 is the dominant subtype in the human myocardium, whereas the rodent heart expresses predominantly SPHK2.[8]

Moreover, we have recently shown significant regional differences in sphingolipid metabolism in the human heart.[5] In patients undergoing coronary bypass graft surgery, papillary muscle of the mitral apparatus was characterized by lower level of ceramide, sphinganine and S1P as compared to the right atrial appendage. On the other hand, expression of enzymes involved in both ceramide synthesis (serine palmitoyltransferase—SPT) and degradation (ceramidases, sphingosine kinases) was markedly higher in the papillary muscle which suggests higher ceramide turnover in the ventricular tissue. However, physiological and pathophysiological consequences of species as well as regional differences in myocardial sphingolipid metabolism remain obscure.

PPARs AS REGULATORS OF SPHINGOLIPID METABOLISM IN THE HEART

Although the role of sphingolipids in the heart has recently attracted much attention, regulation of myocardial sphingolipid metabolism is only poorly investigated. There is, however, some evidence indicating that peroxisome proliferator-activated receptors (PPARs) play a significant role in this process. PPARs are ligand-activated transcription factors of the nuclear hormone receptor superfamily. Three distinct PPAR isoforms termed α, δ and γ have been described. The former two are highly expressed in the heart and are considered to be key transcriptional regulators of fatty acid metabolism in cardiomyocytes.[9] Finck et al[10] demonstrated that high-fat feeding of mice with cardiac-specific overexpression of PPARα leads to accumulation of ceramide in the myocardium. Such effect was not observed in wild type animals which suggests that PPARα may be involved in regulation of myocardial ceramide metabolism. To investigate the mechanism of this phenomenon we examined effects of WY-14643 (a selective PPARα agonist) on sphingolipid metabolism in the rat heart.[11] Activation of PPARα in high-fat fed rats resulted in accumulation of myocardial ceramide and sphingomyelin. This effect was related to stimulation of the de novo sphingolipid synthesis as evidenced by elevated activity of SPT and increased availability of intramyocardial palmitate. Enzymes involved in other pathways i.e., sphingomyelinases and ceramidases were not affected. The mechanism of WY-14643 action on SPT activity is unclear. PPARα stimulation was shown to upregulate expression of SPT in reconstructed human epidermis[12] which suggests involvement of a transcriptional regulation. However, it remains obscure whether PPARα affects SPT expression directly or via changes in cellular lipid metabolism. The latter hypothesis is supported by the fact that in standard chow-fed rats, which did not show accumulation of myocardial free palmitate upon PPARα activation, WY-14643 did not increase either ceramide content or SPT activity.[11] We also observed a modest increase in ceramide content in the heart of rats treated with a selective PPARδ agonist (GW0742) (M. Baranowski, unpublished observation). This increase was accompanied by elevation in the level of sphinganine and long chain fatty acyl-CoA which suggests that the rate of de novo ceramide synthesis pathway was augmented upon PPARδ activation.

Expression of PPARγ in cardiomyocytes is very low and it is the least investigated of all myocardial PPAR isoforms.[9] Several studies demonstrated that thiazolidinediones,

which are selective PPARγ activators, induce expression of glucose transporter 1 and 4 and increase basal and insulin-stimulated glucose uptake in cultured rat cardiomyocytes as well as in the heart of diabetic and insulin-resistant rodents.[13-17] Zhou et al[18] found that administration of troglitazone to Zucker diabetic fatty (ZDF) rats reduced accumulation of myocardial ceramide. This prompted us to examine effect of PPARγ activation on myocardial sphingolipid metabolism. To our surprise, pioglitazone administration increased ceramide level in the heart of rats fed either standard chow or a high-fat diet. However, it should be noted, that this effect was more pronounced in the latter group. A plausible explanation of the discrepancy between our results and those of Zhou et al is that thiazolidinediones lower cardiac ceramide level only in chronically obese and diabetic animals which show excessive lipid deposition in the heart. Accumulation of myocardial ceramide observed in our study was likely a result of its augmented synthesis de novo. This is supported by the fact that administration of PPARγ agonist did not produce evident changes in sphingomyalinase or ceramidase activity, whereas the activity of SPT and the availability of intracellular palmitate was markedly increased in rats fed on either diet. Pioglitazone also induced a modest elevation in the content of SPT protein. However, it did not match the increase in enzyme activity, thus suggesting that the effect of PPARγ agonist was predominantly a result of postranslational modification of SPT protein. It was shown that activity of this enzyme can be modulated independently of changes in the level of its mRNA or protein.[19] However, our data do not exclude a possibility that pioglitazone affects the expression of this enzyme at the transcriptional level. It is widely accepted that increased availability of palmitate induces accumulation of ceramide in cells due to its augmented synthesis de novo.[3] However, in vitro studies on rat astrocytes and pancreatic islets showed that incubation with palmitate also increases activity and expression of SPT.[20,21] These data indicate that palmitate-induced accumulation of ceramide is not solely a result of increased availability of substrate for its synthesis de novo but is also a consequence of activation of the rate-limiting enzyme in this pathway. Therefore, it is likely that the stimulatory effect of pioglitazone on SPT observed in our study was related to accumulation of free palmitate evoked by PPARγ agonist. Collectively, the results of our experiments indicate that PPARs regulate myocardial sphingolipid metabolism predominantly at the level of de novo synthesis (see Table 2). Interestingly, all three PPAR isoforms seem to activate this pathway in the heart, at least in our animal models.

EXERCISE MODULATES MYOCARDIAL CERAMIDE METABOLISM

We found that exercise exerts profound effects on the myocardial content of sphingolipids and affects many pathways of their metabolism.[4] Interestingly, this effect to a large extent depended on exercise duration. We found that 30-minute burst of exercise decreased myocardial content of ceramide. However, its level returned to the baseline after 90 minutes of running and increased further at the point of exhaustion, significantly exceeding the content found in the control animals (Fig. 2). Interestingly, it was recently reported that endurance training consisting of 30-minute exercise bouts reduced ceramide level in the murine heart to a similar extent as observed in our study.[22] Analyses of the activities of enzymes involved in ceramide metabolism revealed that the initial reduction in its content observed in our experiment was likely a result of augmented ceramide degradation, since a concomitant elevation in the activity of acid ceramidase and the level of sphingosine was observed. The subsequent

Table 2. Summary of effects induced by activation of distinct PPAR isoforms on myocardial sphingolipid metabolism

	PPARα		PPARδ		PPARγ	
	Standard Chow	High-Fat Diet	Standard Chow	High-Fat Diet	Standard Chow	High-Fat Diet
Content of sphingolipids						
Sphingosine	↓	↔	↔		↓	↔
Sphinganine	↓	↔	↑		↓	↔
Sphingosine-1P	↔	↔	↑		↔	↔
Ceramide	↔	↑↑	↑		↑	↑↑
Sphingomyelin	↔	↑↑			↔	↑
Enzyme activity						
SPT	↔	↑			↑↑	↑↑
al-CDase	↔	↔			↔	↔
n-CDase	↔	↔			↔	↔
a-CDase	↔	↔			↑	↔
n-SMase	↓↓	↔			↑	↔
a-SMase	↑	↔			↔	↔

PPAR, peroxisome proliferator-activated receptor; SPT, serine palmitoyltransferase; al-CDase, alkaline ceramidase; n-CDase, neutral ceramidase; a-CDase, acid ceramidase; n-SMase, neutral sphingomyelinase; a-SMase, acid sphingomyelinase; ↔, no significant change; ↑, increase; ↓, decrease. Created from data in M. Baranowski et al 2007 J Physiol Pharmacol, M. Baranowski et al 2007 Prostaglandins Other Lipid Mediat and from unpublished results of M. Baranowski.

transition from initial decrease in ceramide content to its accumulation at the point of exhaustion was a consequence of gradual reduction in the activity of acid ceramidase and simultaneous increase in the rate of de novo ceramide synthesis, as evidenced by progressive activation of SPT and accumulation of sphinganine (Fig. 2). This effect could be a result of increased plasma nonesterified fatty acid (NEFA) concentration which occurred during prolonged exercise. As already mentioned in the previous section, in vitro studies showed that incubation of the cells with palmitate increases activity and expression of SPT. Interestingly, in our study the changes in the myocardial activity of SPT reflected those of plasma NEFA concentration.

It should be mentioned that exercise until exhaustion increased not only SPT activity but also content of SPT2 protein (Fig. 2). However, the magnitude of increase in the enzyme activity was over 3-fold higher than that of enzyme protein content. Moreover, SPT2 mRNA level was not affected by treadmill running. Taking together, it indicates that exercise-induced activation of SPT was not a result of its increased expression, but rather a consequence of posttranslational modification of serine palmitoyltransferase protein.

Accumulation of sphingosine is thought to be an important factor contributing to the development of muscle fatigue.[23] We showed previously that exercise until exhaustion induced accumulation of this sphingoid base in rat skeletal muscles.[24] We observed a similar, although much less pronounced, effect also in the heart.[4] There are many reports showing that sphingosine and to a lesser extent also sphinganine, reduce contractility

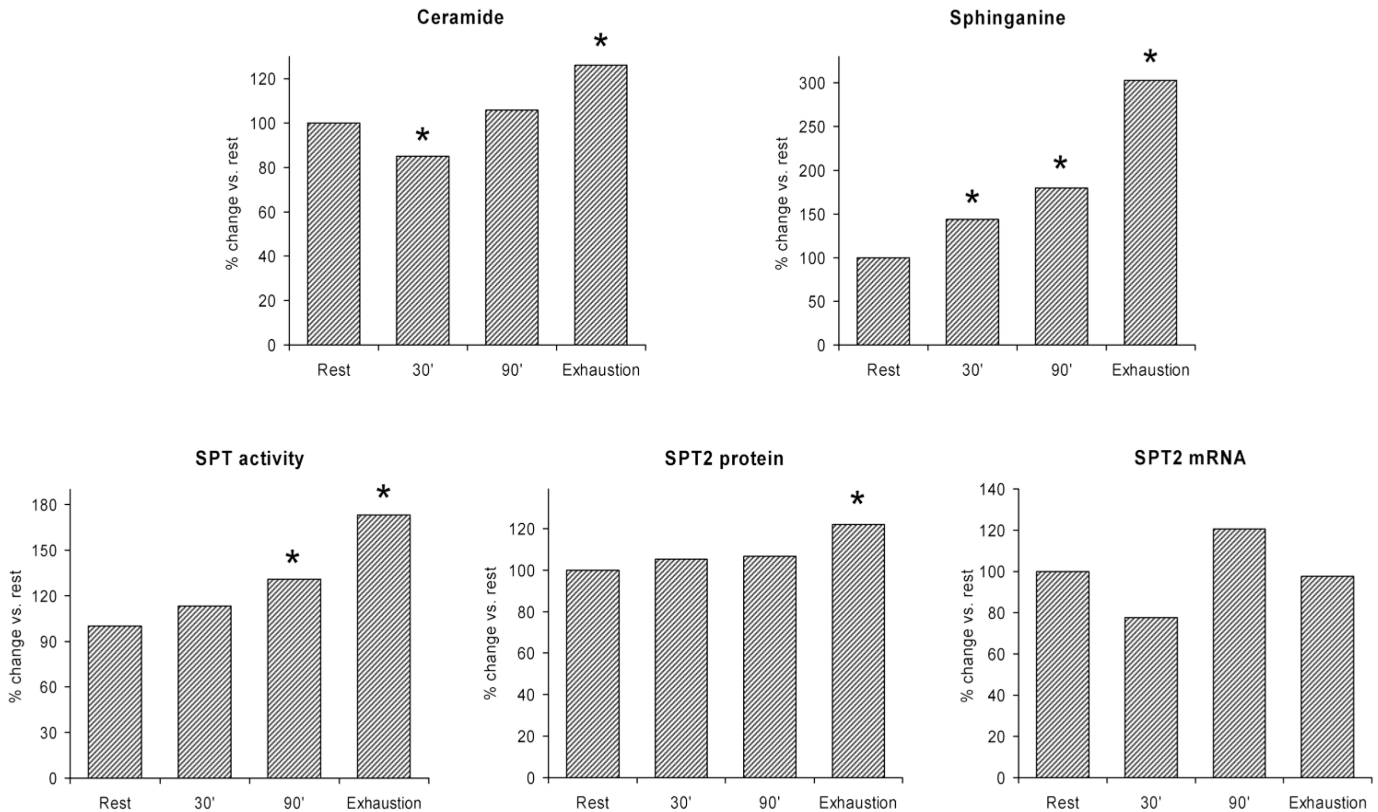

Figure 2. Exercise induces a time-dependent upregulation of the ceramide de novo synthesis pathway in the rat heart. *-statistically significant difference vs rest, SPT-serine palmitoyltransferase, SPT2-serine palmitoyltransferase catalytical subunit. Data redrawn from M. Baranowski et al. Effect of exercise duration on ceramide metabolism in the rat heart. Acta Physiol (Oxf) 2008; 192:519-29.[4]

of cardiomyocytes by blocking calcium release through the sarcoplasmatic reticulum ryanodine receptor.[25,26] It was found that prolonged exercise may induce a reduction in left ventricular systolic function, a phenomenon often called exercise-induced cardiac fatigue.[27] In view of the above data, it is tempting to speculate that accumulation of sphingosine and sphinganine can contribute to the development of fatigue not only in skeletal muscle but also in the heart. However, further studies are required to determine the physiological relevance of this effect.

SPHINGOLIPIDS IN ISCHEMIA/REPERFUSION INJURY OF THE HEART

Krown et al[28] were the first to demonstrate that cell permeable C_2-ceramide induces apoptotic cell death in cardiomyocytes. Shortly after this discovery Bielawska et al[29] using the rat coronary artery occlusion model found that ischemic myocardium was characterized by increased ceramide level that was further elevated upon subsequent reperfusion. This study provided the first evidence that ceramide may be involved in induction of cardiomyocyte apoptosis by ischemia/reperfusion injury. This observation was later confirmed by our and other groups in perfused rat heart and in the rabbit myocardium in vivo.[30-33] However, the mechanism underlying ischemia/reperfusion-induced ceramide accumulation remains unclear. There is some data indicating that increased ceramide production from sphingomyelin is responsible for this effect. Argaud et al[32] found that pretreatment with sphingomyelinase inhibitor—D609 completely prevented ischemia-induced accumulation of myocardial ceramide and reduced cardiomyocyte apoptosis in rabbits. Moreover, depletion of sphingomyelin pool was reported in ischemic/reperfused rat heart.[31] In line with the above observations, hypoxia/reoxygenation was found to rapidly activate neutral (but not acid) sphingomyelinase in neonatal rat cardiomyocytes.[34] Interestingly, pretreatment of the cells with antioxidant prevented both sphingomyelinase activation and ceramide accumulation indicating that increased oxidative stress plays a key role in the effect of reoxygenation on ceramide metabolism in cardiomyocytes. In contrast to the above data, Zhang et al[30] reported inhibition of both neutral and acid sphingomyelinase in the ischemic/reperfused rat heart. Accumulation of ceramide observed in their study was attributed to decreased activity of ceramidase.

We found that ischemic preconditioning (IPC) may afford myocardial protection, at least in part, via modulation of ceramide metabolism.[33] In our experiment, IPC resulted in marked reduction in the ischemia/reperfusion-induced accumulation of ceramide in the perfused rat heart. These results were conformed by Argaud et al[32] who showed that IPC prevented elevation in ceramide content in ischemic rabbit myocardium.

S1P has recently attracted much attention as an important factor protecting the heart against ischemia-reperfusion injury. S1P is not only an intracellular messenger, in fact most of its effects are exerted by binding to a family of plasma membrane G protein-coupled receptors (S1PRs). S1P is normally found in high nanomolar concentrations in human and rodent plasma.[35] Interestingly, this mediator acts also in an autocrine and/or paracrine fashion, as endogenous S1P can be transported to the extracellular space, most likely with the help of cassette transporters.[36] Five subtypes of the S1P receptor have been identified ($S1PR_{1-5}$). Cardiomyocytes express $S1PR_1$, $S1PR_2$ and $S1PR_3$, however, the first subtype was found to be predominant in these cells.[37]

Karliner et al[38] provided the first evidence for cardioprotective effect of S1P. They showed that preincubation of rat neonatal cardiomyocytes with S1P or the ganglioside GM-1, which stimulates endogenous S1P production via activation of SPHK, prevents hypoxia-induced cell death. Cardioprotective action of extracellular S1P and GM-1 was subsequently confirmed in rodent models of ischemia/reperfusion injury.[39,40] The early study by Jin et al[40] suggested that exogenous and endogenous S1P exerts its effects through distinct pathways. They found that cardioprotection afforded by intracellular S1P (generated in response to GM-1) required PKCε, whereas the action of exogenous S1P did not. However, it was recently reported that pertussis toxin (preventing G proteins from interacting with G protein-coupled receptors) as well as the $S1PR_{1/3}$ antagonist abolished GM-1 mediated cardioprotection.[41] These data indicate that intracellulary produced S1P is exported from cardiomyocytes and exerts its protective action via cell surface S1PRs. Therefore, PKCε seems to mediate GM-1 induced SPHK activation rather than action of intracellular S1P. Interestingly, in the perfused rat heart ischemia markedly inhibited SPHK activity and reduced myocardial S1P content, and this effect was maintained over the period of subsequent reperfusion.[42] Consistently, deletion of the SPHK1 gene increased susceptibility of the heart to ischemia/reperfusion injury[43], whereas adenovirus-mediated SPHK1 gene transfer was found to induce protective effect and attenuate postischemic heart failure.[44] It was also suggested that cardioprotective properties of high-density lipoprotein (HDL) involve S1P, as most of plasma S1P is contained within HDL.[45] Interestingly, sphingosine infused at physiological concentrations (high concentrations are cardiotoxic) also exerts protective effect in the perfused rat heart. However, the mechanism of its action is independent of S1PRs and involves cyclic nucleotide-dependent pathways.[46]

There is a controversy as to which S1PR subtype mediates the cardioprotective action of S1P. Experiments made by Karliner's group suggested predominant role of $S1PR_1$. They found that antibody which functions as a $S1PR_1$-specific agonist as well as a synthetic $S1PR_1$ activator protected adult mouse cardiomyocytes from hypoxia to the same extent as exogenous S1P.[47] In addition, the $S1PR_1$ antagonist VPC23019 blocked protection afforded by S1P or its synthetic analog FTY720. The effect of S1P was mediated by a phosphatidylinositol 3-kinase and likely involved activation of Akt/PKB and inhibition of glycogen synthase kinase-3β. However, reports by other groups indicate that $S1PR_2$ and/or $S1PR_3$ rather than $S1PR_1$ mediate the cardioprotective action of S1P. Means et al[48] found that $S1PR_{2/3}$ double knockout mice are characterized by markedly increased myocardial infarct size following ischemia/reperfusion that is accompanied by impaired activation of Akt/PKB. Interestingly, neither $S1PR_2$ nor $S1PR_3$ deletion alone augmented ischemia/reperfusion injury demonstrating some redundancy in S1P receptor function. In addition, Theilmeier et al[49] reported that S1P-mediated cardioprotection was completely absent in $S1PR_3$-deficient mice. It was also abolished by pharmacological nitric oxide synthase inhibition, implicating a crucial role of nitric oxide in this pathway. Recently, Levkau's group demonstrated that mice with cardiac-specific $S1PR_1$ deficiency are susceptible to ischemia/reperfusion injury to the same extent as wild type animals which strongly argues against the involvement of this receptor in S1P-induced cardioprotection.[37] The discrepancy between the results by Karliner's group and those by other investigators may result from differences in experimental models, since protective role of $S1PR_1$ was demonstrated on isolated cardiomyocytes, whereas $S1PR_{2/3}$-mediated cardioprotection was reported in the intact heart. This notion is supported by a recent study by Hofmann

et al[50] who demonstrated that selective $S1PR_1$ agonist exerts protective effect in neonatal rat cardiomyocytes but not in perfused rat heart. In fact, one report indicated that $S1PR_1$ stimulation exaggerates myocardial ischemia/reperfusion injury.[51]

S1P was also found to mediate IPC in the heart. SPHK1 is activated and translocated to the plasma membrane in response to IPC[52] which to a large extent prevents decrease in the enzyme activity and myocardial S1P level upon ischemia/reperfusion.[42] Consistently, pharmacological or genetic inhibition of SPHK1 abolished IPC-induced cardioprotection in the murine heart.[43,52] A recent report by Vessey et al[53] suggests that the mechanism of IPC involves export of produced S1P from cardiomyocytes to the extracellular space and stimulation of $S1PR_1$ and/or $S1PR_3$. Interestingly, it was found that ischemic postconditioning, similarly to IPC, protected isolated mouse hearts against ischemia/reperfusion injury via SPHK1 activation.[54]

We recently found that plasma concentration of S1P in patients with acute myocardial infarction upon admission to intensive heart care unit was markedly lower as compared to healthy controls.[55] Moreover, further reduction in S1P level was observed in these patients within the next five days. Therefore, our data suggest a sustained reduction of the protective effect of plasma S1P after the infarction. However, the mechanism underlying this decrease remains unclear. The main sources of plasma S1P are platelets and erythrocytes.[35] It is, therefore, likely that S1P release from these cells is reduced upon myocardial infarction. However, factors affecting S1P release from blood cells are only poorly recognized. Each patient was subjected to standard antiplatelet treatment, it is then tempting to speculate that this treatment reduced liberation of S1P from thrombocytes. It would be so far unknown and undesired effect of these drugs, especially in view of the recent report showing that exogenous S1P is cardioprotective also in human myocardial tissue.[50]

CERAMIDE AS A MEDIATOR OF LIPOTOXICITY IN THE HEART

Lipotoxicity is the process through which lipid overload leads to cellular dysfunction, cell death and eventually impaired organ function.[56] Accumulation of neutral lipids within cardiomyocytes is a hallmark of the myocardium of humans and rodents with nonischemic heart failure.[56,57] In recent years a hypothesis suggesting that lipotoxicity may contribute to the development of cardiac dysfunction has emerged.[58,59] Direct evidence supporting this notion comes from experiments on mice with cardiac-restricted overexpression of long-chain acyl coenzyme A synthetase 1 (MHC-ACS1),[60] fatty acid transport protein 1[61] and the cell membrane anchored form of lipoprotein lipase (LpL(GPI)).[62] All these transgenic animal models are characterized by increased myocardial fatty acid uptake, lipid deposition in the heart and cardiomyopathy that develops in the absence of disturbances in systemic metabolism or cardiac fatty acid oxidation.

It is still unclear how lipid overload leads to cardiomyopathy, however, there is increasing evidence indicating that accumulation of ceramide plays a key role in this phenomenon. When the balance between fatty acid uptake and oxidation is altered, excess fatty acids is directed towards synthesis of complex lipids, some of which, like ceramide, are toxic. It was found that increase in myocardial ceramide content accompanies cardiac dysfunction in several genetic models of lipotoxic cardiomyopathy (see Table 3). These models include mice with cardiac-specific overexpression of PPARγ, ACS1 and

Table 3. Relationship between myocardial ceramide, cardiomyocyte apoptosis and heart function in various animal models of obesity, diabetes and lipotoxic cardiomyopathy

Experimental Model	Effect on Ceramide Level	Effect on Apoptosis	Effect on Heart Function	Reference
n6-PUFA rich high-fat diet, rats	↔			Baranowski et al. J Physiol Pharmacol 2007
High saturated-fat diet, rat infarct model of heart failure	+ 75%		↔	Rennison et al. Am J Physiol Heart Circ Physiol 2007
High saturated-fat diet, rat infarct model of heart failure	↔		↔	Morgan et al. Am J Physiol Heart Circ Physiol 2005
High saturated-fat diet, rats	↔	↔	↔	Okere et al. Am J Physiol Heart Circ Physiol 2006
n6-PUFA rich high-fat diet, rats	– 39%	↔	↔	
High saturated-fat diet, wild type mice	↔		↔	Finck et al. Proc Natl Acad Sci USA 2003
High saturated-fat diet, MHC-PPARα mice	+ 31%		↓	
High-saturated fat diet, rats	+ 65%	↔		Torre-Villalvazo et al. J Nutr 2009
ob/ob mice	+ 166%	↔		
Streptozotocin-diabetes, rats	+ 14%			M. Baranowski, unpublished observation
Streptozotocin-diabetes, rats	↔		↓	Hayashi et al. Life Sci 2001
Akita Ins2(WT/C96Y) mice (genetic model of Type 1 diabetes)	+ 69%		↓	Basu et al. Am J Physiol Heart Circ Physiol 2009
Zucker diabetic fatty rats	+ 164%	↑	↓	Zhou et al. Proc Natl Acad Sci USA 2000
MHC-PPARγ mice	+ 40%	↑	↓	Son et al. J Clin Invest 2007
MHC-ACS1 mice	+ 230%	↑	↓	Chiu et al. J Clin Invest 2001
LpL(GPI) mice	+ 45%	↔	↓	Park et al. J Lipid Res 2008

PUFA, polyunsaturated fatty acids; MHC-PPARα, cardiac-specific overexpression of peroxisome proliferator-activated receptor α; MHC-PPARγ, cardiac-specific overexpression of peroxisome proliferator-activated receptor γ; MHC-ACS1, cardiac-specific overexpression of long-chain acyl CoA synthetase 1; LpL(GPI), cardiac-specific overexpression of the cell membrane anchored form of lipoprotein lipase; ↔, no significant change; ↑, activation of cardiac apoptosis; ↓, impairment of heart function.

LpL(GPI).[60,63,64] In addition, Zhou et al[18] reported that heart of ZDF rats is characterized by progressive accumulation of ceramide that precedes development of cardiomyopathy. Increased myocardial ceramide content associated with diastolic dysfunction was recently observed also in Akita Ins2(WT/C96Y) mice (a genetic model of nonobese Type 1 diabetes).[65] Interestingly, improvement in cardiac function observed in ZDF rats, Akita Ins2(WT/C96Y) mice and in MHC-ACS1 mice after pharmacological or genetic intervention (administration of troglitazone, insulin and overexpression of diacylglycerol acyl transferase 1 in ZDF rats, Akita Ins2(WT/C96Y) mice and MHC-ACS1 mice, respectively) was associated with decreased myocardial ceramide level.[18,22,65] However, definitive evidence for the key role of ceramide in lipotoxic cardiomyopathy was provided by Park et al[64] They found that inhibition of SPT with myriocin prevented myocardial accumulation of ceramide (but not of other lipids), reduced mortality rate, improved heart function and reduced expression of cardiac failure markers in LpL(GPI) mice. Similar effect was induced by heterozygous deletion of LCB1 gene (encoding one of SPT subunits) which excludes involvement of nonspecific pharmacological effects of myriocin.

Cardiomyocyte apoptosis is one of the mechanisms underlying development of diabetic cardiomyopathy and heart failure.[66,67] Therefore, considering the proapoptotic action of ceramide, its cardiotoxic effects are commonly considered to be mediated primarily by activation of programmed cell death. Accumulation of ceramide was found to be associated with myocardial apoptosis in ZDF rats as well as in mice with cardiac specific overexpression of PPARγ or ACS1[18,60,63] (see Table 3). In addition, interventions leading to a decrease in heart ceramide simultaneously reduced cardiomyocyte apoptosis in ZDF rats and in MHC-ACS1 mice.[18,22] Moreover, in vitro studies on isolated rat cardiomyocytes revealed that incubation of the cells with palmitate induced ceramide accumulation and activation of apoptosis that was attenuated by inhibition of ceramide synthesis.[68,69]

It should be noted, however, that a recent report by Park et al[64] indicates that cardiotoxicity of ceramide may result from its effect on myocardial glucose and fatty acid metabolism rather than induction of apoptotic loss of cardiomyocytes. They used LpL(GPI) mice which were characterized by impaired systolic function, increased rate of fatty acid oxidation and reduced glucose oxidation rate in the heart. Accumulation of myocardial ceramide was observed as well, however, it was not accompanied by cardiomyocyte apoptosis. Interestingly, pharmacological (with myriocin) or genetic (heterozygous deletion of LCB1 gene) inhibition of de novo ceramide synthesis normalized fatty acid and glucose oxidation rate in the heart as well as systolic function. These interventions also corrected the mismatch between myocardial glucose uptake and oxidation, which likely contributed to the development of cardiomyopathy in this model. Park et al also reported that incubation of human cardiomyocyte AC16 cells with C6-ceramide induced downregulation of glucose transporter 4 and upregulation of pyruvate dehydrogenase kinase 4, atrial natriuretic peptide and brain natriuretic peptide gene expression. These changes were consistent with those observed in LpL(GPI) mice that show elevated myocardial ceramide level. The above data strongly suggest that ceramide is able to modulate cardiomyocyte energetic substrate metabolism via transcriptional regulation of the relevant proteins.

In addition, it was recently reported that myocardium of ob/ob mice and rats chronically fed high saturated-fat diet did not show evidence of cardiomyocyte apoptosis

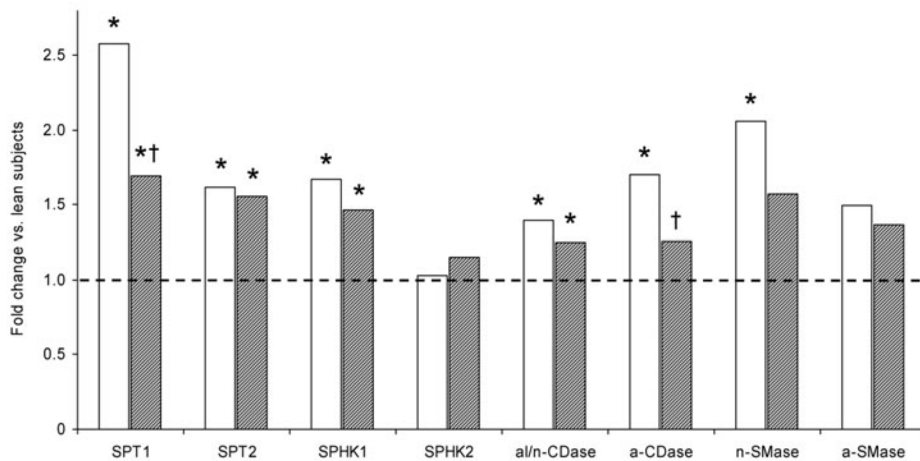

Figure 3. Effect of obesity (open bars) and obesity combined with Type 2 diabetes (hatched bars) on mRNA level of enzymes related to ceramide metabolism in the human heart. *-statistically significant change vs lean subjects, †-statistically significant change vs obese nondiabetic patients, SPT-serine palmitoyltransferase, SPHK-sphingosine kinase, al/n-CDase-alkaline/neutral ceramidase, a-CDase-acid ceramidase, n-SMase-neutral sphingomyelinase, a-SMase-acid sphingomyelinase. Data redrawn from M. Baranowski et al. Myocardium of Type 2 diabetic and obese patients is characterized by alterations in sphingolipid metabolic enzymes but not by accumulation of ceramide. J Lipid Res 2010; 51:74-80.[5]

despite accumulation of ceramide.[70] These data further indicate that increased myocardial ceramide levels do not necessarily lead to activation of apoptosis. Unfortunately, heart function was not assessed in these experiments.

Although data from animal experiments strongly suggest that ceramide may be involved in pathogenesis of heart dysfunction associated with obesity and diabetes, it remains unknown whether similar relationship is present also in the human heart. We attempted to answer this question in our recent study using samples of the right atrial appendage obtained from patients undergoing coronary bypass graft surgery.[5] We found that, compared with lean subjects, myocardium of overweight patients was characterized by marked upregulation of sphingolipid metabolic enzymes expression (Fig. 3). These enzymes included neutral sphingomyelinase, SPT subunits, ceramidases and SPHK1. Interestingly, mRNA level of some genes upregulated by overweight was reduced if concomitant diabetes was present, however, their expression was still higher than in lean subjects (Fig. 3). In addition, we observed elevated DNA fragmentation level (a marker of apoptosis) in the heart of overweight nondiabetic patients that was increased further in overweight Type 2 diabetic subjects. However, to our surprise, there was no accumulation of myocardial ceramide in either group. This was likely due to the fact that cardiac expression of enzymes involved in synthesis and degradation of ceramide was regulated in concert. Our results suggest, that in contrast to rodents, obesity and Type 2 diabetes do not induce ceramide accumulation in the human heart, or at least in the atrium. In addition, ceramide does not seem to be a major factor in cardiomyocyte apoptosis observed in patients suffering from these diseases.

CONCLUSION AND FUTURE PROSPECTS

As reviewed here, evidence from animal models strongly suggest that ceramide accumulation plays a casual role in the pathogenesis of heart dysfunction. However, to date, it remains an open question whether a similar relationship is present also in the human myocardium. Moreover, further research is required to fully elucidate the mechanisms underlying cardiotoxic effects of ceramide. Such information might allow development of new approaches to prevention and treatment of myocardial disease in patients with diabetes and obesity. In contrast to ceramide, S1P plays a critical role in maintaining cardiac cell survival and function. S1P itself as well as agonists of its cognate receptors were found to be potent cardioprotective agents against ischemia/reperfusion injury of the heart in experimental animals. The fact that these compounds are effective also when applied during reperfusion makes them potential candidates for pharmacological postconditioning therapy after myocardial ischemia. Undoubtedly, many new exciting discoveries in the field of myocardial sphingolipid signaling are to be expected in the near future.

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