



The role of dihydrospingolipids in disease

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Abstract

Dihydrospingolipids refer to sphingolipids early in the biosynthetic pathway that do not contain a C4-*trans*-double bond in the sphingoid backbone: 3-ketosphinganine (3-ketoSph), dihydrospingosine (dhSph), dihydrospingosine-1-phosphate (dhS1P) and dihydroceramide (dhCer). Recent advances in research related to sphingolipid biochemistry have shed light on the importance of sphingolipids in terms of cellular signalling in health and disease. However, dihydrospingolipids have received less attention and research is lacking especially in terms of their molecular mechanisms of action. This is despite studies implicating them in the pathophysiology of disease, for example dhCer in predicting type 2 diabetes in obese individuals, dhS1P in cardiovascular diseases and dhSph in hepato-renal toxicity. This review gives a comprehensive summary of research in the last 10–15 years on the dihydrospingolipids, 3-ketoSph, dhSph, dhS1P and dhCer, and their relevant roles in different diseases. It also highlights gaps in research that could be of future interest.

Keywords Adipocyte · Aging · Airway hypersensitivity · Apoptosis · Autophagy · Cancer · Cardiomyopathy · Ceramide · Ceramide synthase · Dihydroceramide desaturase 1-Des-1 · Diabetes · Dihydrospinganine · FB1 toxicity · Hypoxia · Neurodegenerative · Sphingosine kinase · Serine palmitoyl transferase · Sphingosine-1-phosphate—S1P · Sphingosine-1-phosphate receptors · 4-HRP fenretinide

Abbreviations

3-KR 3-Ketosphinganine Reductase
4-HPR *N*-(4-Hydroxyphenyl) retinamideFenretinide
 γ -TE γ -Tocotrienol
ACER3 Alkaline ceramidase 3
ACSL5 Acyl-coenzyme A synthase

ACSL5 Δ 20 acyl-coenzyme A synthase lacking exon 20
ADH Adiponectin hormone
AHA American Heart Association
Akt Protein kinase B
AMPK AMP activated protein kinase
BMI Body mass index
CAD Coronary artery disease
cAMP Cyclic adenosine 3',5'-monophosphate
cDase Ceramidase
cdk2 Cyclin dependent kinase 2
Cer Ceramide
CERKL Ceramide like kinase
CERK Ceramide kinase
CerS Ceramide synthase
CFTR Cystic fibrosis transmembrane conductance regulator
COX-2 Cyclooxygenase 2
CRF Cardiorespiratory fitness
CTGF Connective tissue growth factor
CVD Cardiovascular disease
Des1 Dihydroceramide desaturase 1
Des2 Dihydroceramide desaturase 2

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DhCer	Dihydroceramide	S1P	Sphingosine 1 phosphate
DhSph	Dihydrosphingosine/Dihydrosphinganine	S1PP	Sphingosine 1 phosphate phosphatase
DhS1P	Dihydrosphingosine 1 phosphate/dihydrosphinganine 1 phosphate	S1PR1–5	Sphingosine 1 phosphate receptor 1–5
EAP	Ethanolamine phosphate	SPL	Sphingosine 1 phosphate lyase
ER	Endoplasmic reticulum	SPT	Serine palmitoyltransferase
ERK	Extracellular signal regulated kinases	SPLTC1	Serine palmitoyltransferase long chain base 1
FAK	Focal adhesion kinase	SPLTC3	Serine palmitoyltransferase long chain base 3
FB1	Fumonisin B 1	STEMI	ST-segment elevation myocardial: infarct
FFA	Free fatty acid	SCC19	Squamous cell carcinoma cell
HDAC2	Histone deacetylase 2	T2DM	Type 2 diabetes mellitus
HDL	High density lipid	TNF- α	Tumour necrosis factor α
HepG2	Human hepato-carcinoma cell	VEGF	Vascular endothelial growth factor
HIF1- α	Hypoxia inducible factor 1- α	WC	Waist circumference
HOMA-IR	Homestasis model of insulin resistance		
HSP27	Heat shock protein 27		
HUVEC	Human umbilical endothelial cell		
FTY720	Fingolimod		
IL-1	Interleukin 1		
IL-6	Interleukin 6		
JNK	c-Jun N terminal kinase		
LDL	Low density lipid		
LPS	Lipopolysaccharide		
LRS	Lipidomic risk score		
MAPK	Mitogen activated protein kinases		
MI	Myocardial infarct		
MnTBAP	Manganese(III) tetrakis (4-benzoic acid) porphyrin		
MTORC1	Mammalian target of rapamycin complex 1		
NADH	Nicotinamide adenine nucleotide		
NADPH	Nicotinamide adenine nucleotide phosphate		
NAFLD	Non-alcoholic fatty liver disease		
NFATC	Nuclear factor of activated T cells		
NK-k β	Nuclear factor kappa light chain enhancer of B cell		
Nrf2	Nuclear factor erythroid related factor 2		
PDGF	Platelet derived growth factor		
PDT	Photodynamic therapy		
PeIF2 α	Phosphorylated eukaryotic translation initiation factors 2 α		
PERK	PKR like endoplasmic reticulum kinase		
PKC α	Protein kinase C α		
PLD	Phospholipase D		
PPAR γ	Peroxisome proliferator-activated receptor γ		
RAR	Retinoic acid receptor		
RMC	Renal mesengial cell		
ROS	Reactive oxygen species		
S6K	Ribosomal protein S6 kinase		
SAFHS	San Antonio Family Heart Study		
SEK-1	Dual specificity mitogen activated protein kinase kinase 1		
SD	Sprague Dawley		
SK 1 and 2	Sphingosine kinase 1 and 2		

Background

Since their discovery in the 1800s, sphingolipids have been shown to play key roles in physiological and pathological states by functioning as mediators or effectors of cellular signals. They are integral components of all eukaryotic cell membranes. It is now known that sphingolipids play a role in cell apoptosis, autophagy, oxidative stress and inflammation [1, 2, 3] and in disease states such as cancer, multiple sclerosis and diabetes [4, 5, 6]. These cellular events are effected through activation and interaction of the sphingosine 1 phosphate receptors (S1PR1–5), enzymes such as sphingosine kinases (SK 1 and 2), ceramide synthases (CerS1–6) or sphingolipids such as sphingosine 1 phosphate (S1P), and ceramides (Cers) [7]. Accordingly, there is significant interest in targeting the enzyme of sphingolipid metabolism and S1PRs in the discovery of new therapies. The term sphingolipids extends to a lot of other lipids and enzymes within the sphingolipid de novo biosynthesis pathway (Fig. 1). These include 3-ketoSph, dhSph, dhS1P and dhCer, as well as enzymes such as serine palmitoyltransferase (SPT), dihydroceramide desaturases (Des 1 and 2), and ceramidases (CDases). Here we, attempt to give a comprehensive review of literature focusing on the evidence for the role of the aforementioned dihydrosphingolipids in relevant disease states and the associative effects they may have or the possible roles they may play. The information presented in this review was derived through data searches in Ovid, Medline and Embase using the MeSH terms (dihydrosphingosine 1-phosphate, sphinganine 1 phosphate, 3-ketosphinganine, dihydroceramide, dihydrosphinganine and dihydrosphingosine) and keyword searches of the same. The articles derived from the search were limited to human and animal studies and the English language. It is hoped that this review will also shed light on much needed areas of research on the relevance of dihydrosphingolipids and their roles in diseases.

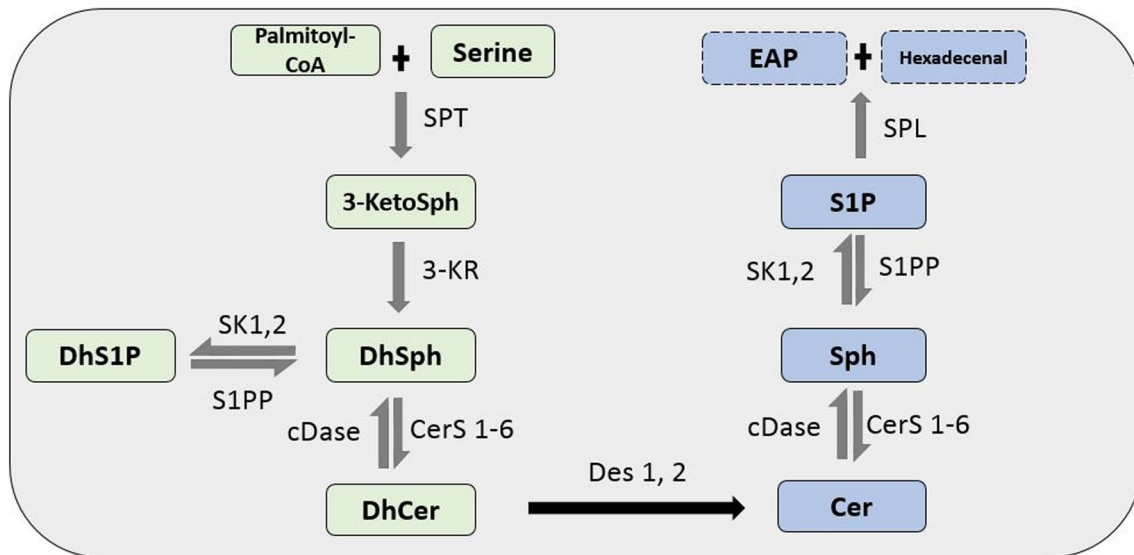


Fig. 1 De novo sphingolipid biosynthesis pathway. In the de novo pathway, the condensation of palmitoyl-CoA and serine by the enzyme SPT forms 3-ketoSph. This is then reduced by 3-KR to dhSph. The acylation and phosphorylation of dhSph by CerS1-6 and SK 1 and 2 leads to the formation of dhCer and dhS1P, respectively. Des-1 and -2 then catalyze the desaturation of dhCer to Cer, which is

a non-reversible reaction. The metabolization of Cer by CDase produces Sph. The production of S1P from Sph is exclusively phosphorylated by SK 1 and 2. S1P is then degraded to ethanolamine phosphate (EAP) and trans-2-hexadecenal by S1P lyase (SPL). DhS1P and S1P can be converted back to dhSph and Sph by S1P phosphatase (S1PP) and dhSph and Sph to dhCer and Cer, respectively, by cDase

De novo synthesis of sphingolipids

Briefly, apart from the de novo synthesis pathway (Fig. 1), sphingolipids are also synthesized through the salvage pathway and the sphingomyelin pathway. Regulation of plasma levels of sphingolipids generally occurs through the de novo synthesis pathway [8]. The backbone of the sphingolipids are Sph and dhSph, which are composed of an amino alcohol, from which all the other sphingolipids are derived by the enzymatic activity of a number of enzymes along the pathway. Most of the enzymatic activities along the de novo synthesis pathway are reversible except a few, including the conversion of dhCer to Cer. Thus, the enzymes responsible for this, Des-1 and -2, have now been described as gatekeepers [9].

The segment of the pathway that begins at Des-1 and -2 to S1P which includes Cer, Sph and S1P has been studied the most and their relevance in disease is well documented by other reviewers [10–13]. Therefore, in this review, the focus is on highlighting dhCer, dhS1P, dhSph and 3-ketoSph; dihydrospingolipids; and the possible regulatory and contributory effects of these dihydrospingolipids in diseases.

DhCer in disease

Overview and structure

DhCers lack the C4-double bond observed in Cers (Fig. 2); however, they also serve as precursors of complex sphingolipids such as dihydrospingomyelins and dihydrogangliosides. For years, dhCers were thought to be biologically inactive due to them being less abundant, compared to Cers. This perception changed with the development of fenretinide [*N*-(4-hydroxyphenyl)retinamide]-(4-HPR), which was found to inhibit Des-1 by the Merrill Group [14]. Des-1 is found in all tissues, whereas Des-2 has been found in skin, intestine and kidney [15]. A later study further showed that the ablation of Des-1 and 2 shifts sphingolipid synthesis pathway toward the sphingolipid lacking the double bond introduced by Des-1 and -2, such as dhS1P,

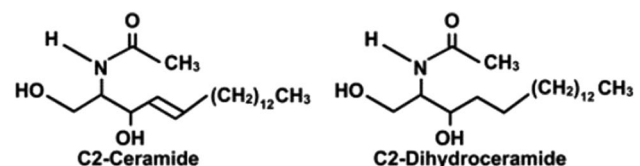


Fig. 2 Comparison of C2-ceramide with C2-dihydroceramide, without the double bond

dhSph, dhsphingomyelin (dhSM) and especially dhCer [16]. Together, these discoveries led to new functional discoveries for dhCers in apoptosis, autophagy, hypoxia and cell proliferation, as reviewed by Siddique et al. [17].

In the conventional sphingolipid synthesis pathway, dhCers are produced as a result of the addition of fatty acyl-CoAs of differing chain lengths to dhSph by the enzyme CerS. The six isoforms of CerS expressed in mammals are encoded by different chromosomes and exhibit preference for a defined chain length of fatty acyl-CoA [18], therefore portraying different functional, structural and biochemical attributes [19]. The dhCer chain lengths that are mentioned in this review are summarized in Table 1, except for the studies in cancer cells. It should be noted that most of the studies referenced in the table also had alterations in the Cer levels; however, they have not been mentioned due to the focus of the review in highlighting dhCers and the other dihydro sphingolipids. The majority of the studies in which dhCer has been mentioned, from 1990s to 2009, used cell penetrant dhCer bearing short acyl chains as negative controls in experiments tailored toward elucidating the effects of Cers in biological systems or disease conditions [20–22]. Due to the way in which these were used, most reported no effects and thus will not be included here. However, evidence contained in more recent studies paint a different picture of longer chain dhCers in terms of diseases.

DhCer in brain diseases

Research on sphingolipids in the brain has focussed on the glycolipids which include the cerebrosides, gangliosides and ceramide oligosaccharides as well as on Cers. Though Sun et al. [60] give a comprehensive review of the role of sphingolipids in stroke, the review does not highlight dihydro sphingolipids, which may be due to most of the studies focussing on other sphingolipids. Here, we highlight studies that have mentioned dhCer levels in brain-related diseases.

A study investigating the effects of hypoxia on sphingolipid metabolism in human cerebral endothelial cells found that dhCers (long chains) were increased together with other sphingolipids [23]. In addition, increased dhCer levels were also seen after subarachnoid haemorrhage [24]. Both of these studies allude to the involvement of dhCer in the mechanisms of disease in oxygen deprivation states such as stroke. Not only this, but dhCer levels have also been noted to be altered in studies related to certain neuronal diseases such as leukodystrophia [26], Alzheimer's [61], Huntington's disease (HD) [62] and in episodic migraines [25]. Though the cause of migraines is not so clear, genetic anomalies in the enzymes could have played a part in the reduced levels of dhCer seen in the migraine study, as shown by Matesanz et al. [63]. This study hypothesized that the splice variant of the acyl-coenzyme A synthase 5 (ACSL5)

gene which lacked exon 20 (ACSL5- Δ 20), could have led to the decrease in CerS, and thus dhCer levels. On the other hand, genetic mutations in other enzymes such as ACER3, which is an alkaline ceramidase (CDase), have been linked to elevated dhCer (C18:1 and C20:1) levels observed in the plasma of childhood leukodystrophic twin patients with a genetic mutation at p.E33G, responsible for the catalytic activity of ACER3. In Alzheimer's disease, the inhibition of the gatekeeper enzyme, Des-1 by XM461 and XM462, increased dhCer levels in Alzheimer's transgenic mice, which led to the induction of autophagy and reduced amyloid secretion by neuronal cells through loss of ribosomal protein S6 kinase (S6K) activity due to reduced mammalian target of rapamycin complex 1 (MTORC1) activity [64]. The autophagy effect exerted by increased dhCer observed in this study is corroborated by studies in cancer cells that have shown similar effects [65, 66]. However, clinically, others have shown that increased plasma dihydro sphingomyelin/dhCer and sphingomyelin/Cer ratios are predictive of slower progression among Alzheimer's disease patients [61]. In addition, reduced dhCer (C18:0) including dhSph and dhS1P levels and mRNA expression of the enzymes CerS1 and serine palmitoyltransferase long chain base 1 (SPLTC1) have been observed in transgenic mice brains manifesting HD. These reductions may be a result of the reduced level of SPLTC1, which impacts the entire de novo sphingolipid synthesis pathway. These studies show an association of dhCer with the progression of degenerative brain diseases as well as in other brain-related diseases, which makes it a potential target as a biomarker. There are also conceivable genetic associations of the enzymes in the sphingolipid pathway with neurodegenerative diseases. However, whether or not dhCer has a causal effect is an area that warrants further research.

DhCer in diabetes

It is now known that dyslipidaemia commonly occurs in diabetes [67], which is a major risk factor for developing cardiovascular diseases (CVDs) [68]. The main characteristic of dyslipidemia in diabetes is high triglyceride levels, reduced high-density lipids (HDL) and slightly elevated low-density lipids (LDL)-cholesterol, with a dominance of the atherogenic small dense LDL [69]. Studies have shown that the sphingolipid, S1P, is bound to HDL in plasma and its distribution is shifted to other non-HDL carriers in the plasma, when HDL levels are low [70]. A number of studies also support the role of Cer and Cer16:0 in particular, in insulin resistance and glucose intolerance [71–74]. These evidences show that sphingolipid metabolism and transport, including dhCer, can be altered in diabetes affecting insulin resistance and mitochondrial and adipose tissue homeostasis.

Table 1 Summary of the dhCer acyl chain length-specific effects in the different pathologies mentioned in the review

Effects	Study type	Type of cell, animal or sample	Pathways involved	dhCer acyl chain length	Levels	References
Brain disease and dhCer						
Hypoxia	In vitro	Cerebral endothelial cells		Long chain (unspecified)	↑	[23]
Subarachnoid haemorrhage	Human	CSF		Unspecified	↑	[24]
Episodic migraine	Human	Plasma		Very long chain (unspecified)	↑	[25]
Leukodystrophia	Human	Plasma		Unspecified	↑	[26]
Diabetes, aging and dhCer						
Insulin inhibition by palmitate	In vitro	C2C12 muscle cell	Inhibition of Akt/PKB pathway	Unspecified	↑	[27]
Gluco-lipotoxicity	In vitro	β-Islet cells		C16:0, C18:0, C22:0, C24:1	↑	[28]
	In vitro	Isolated β-islet cells		C16:0	↑	[29]
Reduced mitochondrial respiration	In vitro	C2C12 myotubes		Long chain (unspecified)	↑	[30, 31]
Cer channel formation in mitochondria	In vitro	Isolated mitochondria		C16:0, C2:0	↑	[32]
Increased dhCer	In vitro	Mature adipocytes	Nutrient stress pathway involving AMPK	Unspecified	↑	[33]
Increased insulin sensitivity by overexpressing adiponectin	In vitro	Rat single muscle cells		C20:0	↓	[34]
Inhibition of plasma insulin signalling and amino acid transport	In vitro	Primary human trophoblast cells		Unspecified	↑	[35]
Insulin resistance in high fat and high fructose diet	In vivo	Rhesus monkeys		Unspecified	↑	[36]
	In vivo	Rat single muscle cells		C20:0	↓	[37]
Insulin resistance due to adiposity	In vivo	Lactating cows		Unspecified	↑	[38]
Associated with BMI and increased waist to hip ratio	Human	Plasma		Unspecified	↑	[39, 40]
Associated with higher plasma cholesterol and statin use	Human	Plasma		C20:0	↑	[40]
Correlated with waist circumference	Human	Plasma		C18:0, C20:0, C22:0, C24:1	↑	[41]
Obesity and type 2 diabetes	Human	Plasma		C24:1, C18:0	↑	[42–44]
Increased insulin sensitivity due to diet and exercise and antidiabetes therapy	Human	Plasma		Unspecified, C18:0, C24:1	↓	[45, 46]
Associated with lower cardio-respiratory fitness in older adults (< 55 years)	Human	Plasma		C20:0	↑	[47]
Hypoxia	In vivo	Right ventricles of rats		C16:0	↑	[48]
Cardiovascular disease and dhCer						
Induced apoptosis in the presence of high concentrations of saturated fat	In vivo	Cardiac myocytes		C16:0	↑	[49]

Table 1 (continued)

Effects	Study type	Type of cell, animal or sample	Pathways involved	dhCer acyl chain length	Levels	References
Coronary artery disease Lung disease and dhCer	In vivo	Atherosclerotic plaques	Induced IL-6	Unspecified	↑	[50, 51]
Cystic fibrosis	In vitro	Lung epithelial cells		C16:0	↑	[52]
Effect of inhibition of CerS by FTY720	In vitro	Human lung epithelial cells	Defective expression of CFTR gene	Unspecified	↓	[53]
Emphysema, autophagy in hypoxic state, decreased lung cell proliferation	In vivo	SD rat lung, lung epithelial cells	Reduced HIF-1 α and VEGF protein expression	Unspecified	↑	[54] [55]
Liver disease and dhCer						
Inflammation	In vitro	HeG2 Cells		Unspecified	↑	[56]
Knock down of Des-1	In vitro	Huh7 hepatocyte cells		Unspecified	↑	[57]
Raised in hepato cell carcinoma than hepatic cirrhosis	Human	plasma		Unspecified	↑	[58]
Raised in non-alcoholic fatty liver disease	Human	plasma		C16:0, C18:0, C24:0, C24:1	↑	[59]

Insulin resistance

Insulin resistance is an important factor in type 2 diabetes and pre-diabetes [75], while chronic exposure to free fatty acids (FFA), such as palmitate, causes insulin resistance. In cellular models (C2C12 myotubes and isolated β -islet cells) of insulin resistance induced by palmitate, increased dhCer (C16:0), Cer (C16:0) and dhSph have been noted [27, 29]. The study in C2C12 myotubes also indicated the inhibition of the Akt/PKB pathway in promoting the insulin resistance [27], which is similar to how Cer has been shown to antagonize insulin signalling [72]. Others have shown that palmitate causes an increase in specific dhCer (C16:0, C18:0, C22:0, C24:1) and Cer chain lengths, resulting in glucolipotoxicity in beta cells [28]. These studies denote the changes in dhCer as associative effects, rather than a causal effect. There are recent studies which imply that dhCer and the de novo sphingolipids could have an additive effect to that of Cers. For example, Reali et al. [71] showed in their model of *ob/ob* mice macrophage that increases in the enzymatic activity of CerS6 led to increased Cer C16:0 and that the impairment of insulin signalling in these model occurred at 16 weeks when the levels of all the sphingolipids were upregulated. This increase in all sphingolipids provides a link to the clinical [76] and animal [36] studies that have shown increases in both dhCer and Cer levels. This is further supported by findings that the enzymes SPT, CerS and Des-1 are not specific to one type of sphingolipid in their sensitivity but quite diffuse [71], implying that they contribute towards balancing the regulation of sphingolipids. Perhaps, this is one of the reasons for the insignificant changes in Cer levels seen in the same cohort of patients

with significant increase in dhCer levels [76]. Moreover, the type of abundant saturated fats available in the system could also determine the type of dhCer species produced. For example, when SPT is induced by high saturated fats, it has been shown to switch substrate specificity (palmitate to myristate), producing different dhCer C16:0 species [49]. In terms of therapy, increasing the expression of adiponectin receptors in single muscles of rats fed a high fat diet did increase the insulin sensitivity and also reduced the level of dhCer and Cer [77], which may be occurring through the adiponectin/AMP-activated protein kinase (AMPK) pathway. Activation of the adiponectin-AMPK pathway leads to inhibition of manoyl-CoA resulting in the increase of carnitine palmitoyltransferase 1 (CPT1), the rate-limiting step in fatty acid oxidation [78]. Furthermore, two other studies have also noted increase in the levels of dhCer and Cer in primary human trophoblasts (PHT) [35], and in cows transitioning from gestation to lactation [38], implicating these sphingolipids in gestational diabetes.

These studies show that the changes in dhCer levels in lipid-driven insulin signalling are directly related to it being a precursor to Cers and that Cer is involved in insulin resistance. However, it should be noted that these studies were aimed at Cer; therefore, the question of the effect that dhCers has on insulin signalling still remains unanswered.

Mitochondrial homeostasis

The clinical complications associated with type 2 diabetes such as dyslipidaemia, hyperglycaemia and insulin resistance are linked to mitochondrial defragmentation [79]. Mitochondrial homeostasis is maintained through a balance

of fusion and fission, mitochondrial biogenesis and degradation. Increased longer chain dhCer due to ablation of *Des-1*^{-/-} in mouse embryonic fibroblasts and Des-1 inhibition in C2C12 myotubes reduced mitochondrial respiration and complex IV (cytochrome c oxidase) expression in the presence of lipopolysaccharides (LPS) [31, 80]. Complex IV catalyses the final step in the mitochondrial electron transfer chain and is thought to be a major regulation site for oxidative phosphorylation [30]. A reduction in complex IV would impair ATP synthesis. Introduction of LPS to the C2C12 myotubes caused an increase in Cers and had opposite effect to dhCers. LPS also increased oxidative stress and mitochondrial fission through dynamin-related protein 1 (DRP1) which was inhibited when SPT was inhibited by myriocin. Increase in DRP1 and oxidative stress leads to increased mitochondrial defragmentation and insulin resistance [81]. Another study has shown that dhCer (C2, 95% and C16, 51%) can inhibit Cer channel formation in mitochondria [32], inhibiting apoptosis. The study in mouse embryonic fibroblasts also found the *Des-1*^{-/-} cells to be resistant to apoptosis through the Akt/PkB pathway, but had increased autophagy through AMPK activation as a result of the impaired ATP synthesis. These studies show that dhCer can disrupt the processes of mitochondrial biogenesis and degradation, and contribute towards improving mitochondrial function by increasing autophagy and decreasing apoptosis, inhibiting mitochondrial respiration and possibly inhibiting DRP1 and oxidative stress.

Adipose tissue homeostasis

A number of researchers have shown how the selective manipulation of Des-1 and its substrates may be a physiologically advantageous strategy to improve adipose tissue homeostasis and ameliorate the burden of obesity-associated metabolic complications. For example, Barbarroja et al. [33] showed that an ablation in expression of Des-1 or the pharmacological inhibition of Des-1 in 3T3-L cells led to an increase in dhCer/Cer ratio with concurrent increases in oxidative stress, cell death and inhibition of cell differentiation. Their results also showed an increase in the protein expression of GLUT4, which facilitates the uptake of glucose from the plasma. Moreover, 5- to 16-fold increases in dhCer with activation of p38-MAPK, protein phosphorylated eukaryotic translation initiation factor 2 α (PeIF2 α) and autophagy markers (Beclin1 and LC3B II) have been observed in mature adipocytes treated with 4-HPR-fenretinide [34]. PeIF2 α is involved in the nutrient stress response pathway, which has been shown to contribute to the pathogenesis of diabetes. In this study, 4-HPR-fenretinide was shown to utilize both retinoic acid receptor (RAR)-dependent and -independent pathways to regulate adipogenesis and prevent obesity in mice fed a high fat diet.

The RA-dependent pathway results in increased Cer despite the presence of 4-HPR-fenretinide, an example of which is given by Bikman et al. [37]. 4-HPR-fenretinide is a structural derivative of retinoic acid, and research in cancer cells has also shown that this compound and dhCer are associated with the activation of cellular stress responses and induction of autophagy [65, 82]. In fact, a recent study in kidney cells has shown that 4-HPR-fenretinide induced polyubiquitination of Des-1, which exhibited “gain of function” and activated pro-survival pathways, p38 MAPK, JNK and X-Box Protein-1s [83]. In addition, dhCers directly suppressed the transcriptional activity of peroxisome proliferator-activated receptor gamma (PPAR γ) similar to that seen in *Degs1* (*Des-1* regulatory gene) ablation, which also suppressed cyclins (D1, D3 and E) and cyclin-dependent kinase 2 (cdk2), thus impairing adipocyte programming in pre-adipocytes [33]. PPAR γ plays a central role in adipogenesis and lipid metabolism [84]. We would like to note that the inhibition or ablation of Des-1 led to feedback inhibition and downregulation of SPLTC1 and CerS6, a systemic counter balancing mechanism which could be triggered by the increased dhCer levels.

These studies showed that dhCer could be involved in the disruption of adipogenesis and cause cell death either as a direct result of Des-1 inhibition or by itself. Since the inhibition of Des-1 certainly leads to dhCer accumulation, it is possible that it disrupted adipogenesis early on through inhibition of PPAR γ transcription, which is necessary for the terminal differentiation of the adipocytes, and the increased oxidative stress and cell death through autophagy can be attributed to dhCer. However, whether it functions as a ligand or has lipid–protein interactions or lipid–enzyme interactions is elusive since these studies focussed on Des-1.

Epidemiological findings

Epidemiological studies aimed at decoding the associations between sphingolipids and known risk factors [42, 43, 85] or markers for diabetes [39, 40] show increases in dhCer to be precedent of increases in Cer, with concomitant reductions seen when diet, exercise and anti-diabetics are introduced [45, 46]. While others found it to have no longitudinal or cross-sectional association with pre-diabetes or diabetes, Cer (C18:0, C22:1) did [39]. This can be attributed to the progression of the de novo synthesis pathway towards Cer. However, there are at least two studies which show dhCer levels to be opposite to that of Cers. One study found dhCer to be genetically correlated with waist circumference [41], while Cer was not, even after adjusting for confounders such as age and sex, and accounting for genetic differences by using polygenic models. The other study found dhCer to be elevated in the abdominal adipose tissue of obese and non-obese diabetics when compared to lean non-diabetics

[39, 41, 86], with negative correlation between homeostasis model of insulin resistance score (HOMA-IR) and Cer. It is possible that sampling differences (plasma vs. adipose tissue from abdominal area) could account for the differences; however, the latter study did not adjust for patients taking the anti-diabetic metformin, which could have had an effect on the HOMA-IR scores. Despite these, there is evidence for dhCer to be used as a predictor for developing type 2 diabetes. A study showed dhCer C18:0 to be the single best predictor for progression to diabetes, with those progressing from non-diabetic to diabetic within 10 years having higher dhCer C18:0 at baseline [87]. Furthermore, researchers in the USA have recommended that the lipidomic risk score (LRS) assessment criteria—dhCer (C18:0) included in the criteria—be used in conjunction with metformin supplementation for individuals with high risk of developing type 2 diabetes [88]. The LRS score predicted future type 2 diabetes independently of prediabetes with an accuracy of 76%. Therefore, dhCer lipid profiling in obese patients could be a tool for predicting the onset of pre-diabetes and diabetes in this population.

In summary, apart from the epidemiological evidence showing its value as a predictor for developing type 2 diabetes, the *in vitro* and *in vivo* studies show a possible therapeutic potential in targeting the Des-1 enzyme and elevating dhCer, which could increase autophagy, reduce adipogenesis and lipid accumulation, leading to increased insulin sensitivity and glucose uptake as summarized in Fig. 3.

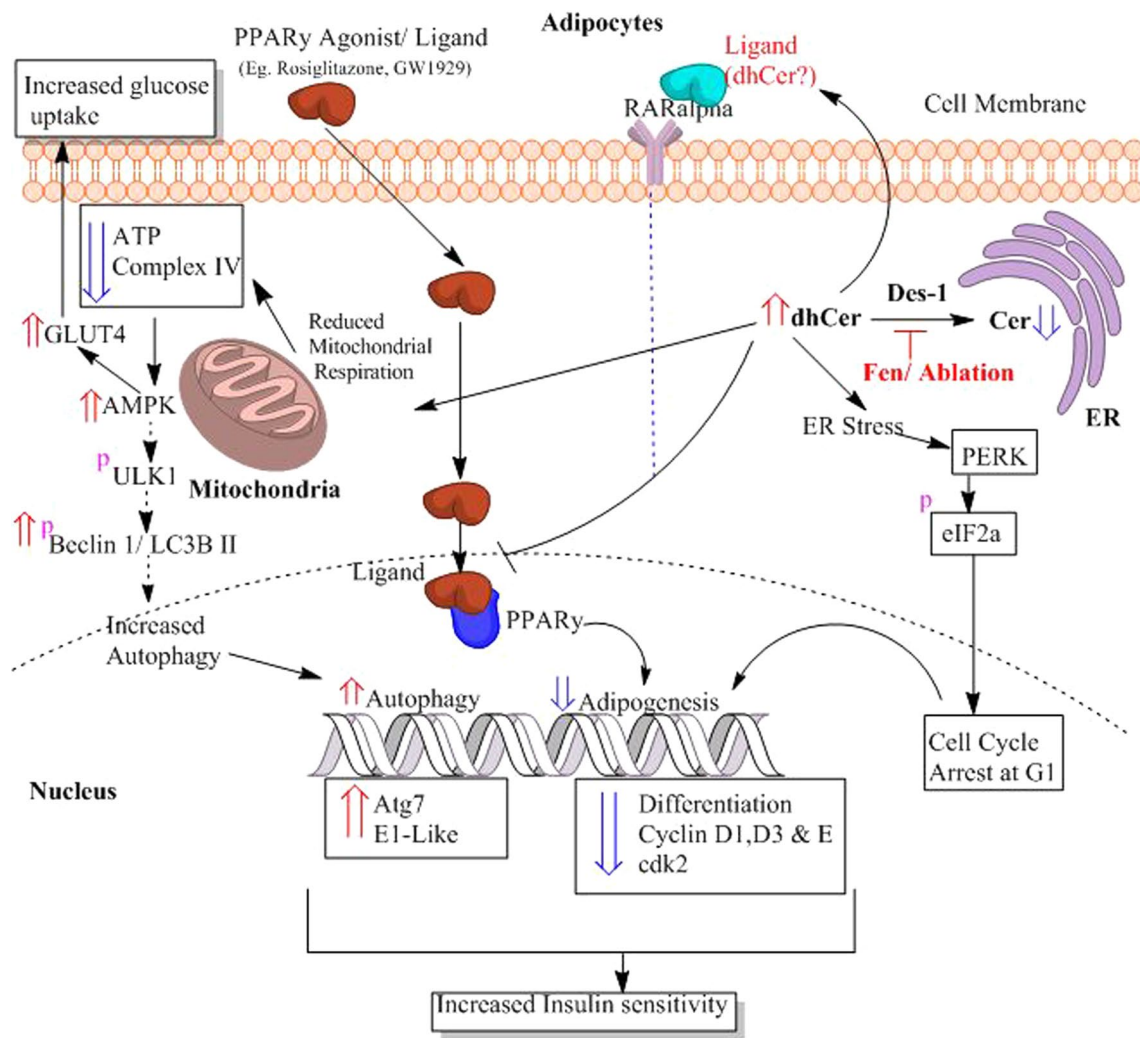
DhCer in aging and disease

As age increases, lipid dysregulation increases also and gives rise to the risk of developing CVDs. A current epidemiological report released by the American Heart Association (AHA) highlighted that 48.6% of adults aged ≥ 40 years in the USA are eligible for statin “lipid-lowering” therapy [89]. Chronological aging has a tremendous effect on cardiorespiratory fitness (CRF) and low levels are representative of risk factors for CVDs, dyslipidaemia and hypertension [90–92]. CRF refers to the ability of the cardiac and respiratory systems to supply oxygen to skeletal muscles during sustained physical activity. Increased C20:0 dhCer was found to be strongly associated with lower CRF in both men and women aged 54–96 years [47], while C24:0 dhCer was not. This connection of dhCer to hypoxia is supported by evidence in mice hypoxia models, which showed elevated levels of dhCer C16:0 in the right ventricles [48] and in the heart [93] from week 4 to week 8, with a concomitant decrease in Cer and expression of Des-1. The latter study identified that the Des-1 promoter harbours overlapping sites for *HAND2* and nuclear factor of activated T cell (NFATC) transcription factors, which have been shown to be important in the development of cardiac systems. Both of these factors were

required for upregulation of Des-1, while the re-activation of *HAND2* in failing hearts due to co-operation between NFATC and miRNA-125 has been shown to aid cardiac dysfunction [94]. Whether the hypoxia-induced dhCer is a protective mechanism even in reduced CRF through autophagic flux remains to be answered. Furthermore, increased local dhCer levels were shown to be associated with reductions in thymocyte apoptosis and age-associated thymic involution in aged mice, when growth hormones were introduced [95]. This most likely fostered autophagy in thymic epithelial cells, which shapes the T cell repertoire and tolerance. These contrasting effects of hypoxia and autophagy point to tissue-specific associations of dhCer. However, this remains inconclusive due to the lack of evidence with regard to dhCer in aging. Therefore, including dhCer and dihydrosphingolipids in future lipidomic profiling studies in the elderly should be encouraged.

DhCer in cardiovascular disease

Though cholesterol is vital for healthy bodily functions, excess amounts in the blood due to increased dietary intake of saturated fats can lead to buildup of atherosclerotic plaque and coronary artery disease (CAD), increasing the risk for heart attacks. Cers are known to be associated with cholesterol in terms of lipid rafts formation [96], which serve as the basis for signal transduction during inflammatory responses. In human atherosclerotic plaques [50] and rat models of hypercholesterolaemia [97], dhCers were found to be increased. Both dhCer and Cer correlated with the release of the inflammatory cytokine interleukin 6 (IL-6), but only dhCer correlated with macrophage inflammatory protein 1 β (MIP-1 β) release [50]. Elevated IL-6 levels in atherosclerosis results in effects on endothelial cells (activation), platelets (prothrombotic effect), muscle cells (proliferation) and macrophages (lipid accumulation) that are involved in lipid processing and plaque formation [98], while increased MIP-1 β (also known as chemokine CC motif ligand 4—CCL4) in patients was linked to atherosclerosis and plaque instability [99]. What role this increase in dhCer plays in plaque stability is still debatable, since the extracellular addition of dhCer to human aortic smooth muscle cells did not cause apoptosis, whereas Cer did [50]. Apoptosis of cells in the vessel walls increases plaque instability. Apart from these CAD-related studies, dhCer levels have also been found to be elevated in patients with rheumatoid arthritis [100], patients with “HeartWare” left ventricular assist devices [101], hypertensive rats [102] and in doxorubicin-induced cardiac toxicity [103]. These studies point to the possible role of dhCer as a marker for cardiac pathology. The correlation between MIP-1 β and dhCer should also be investigated further, since MIP-1 β is also implicated in type 2 diabetes. However, there is a lack of mechanistic studies



Double blue arrow- decrease, double red arrow- increase, p- phosphorylation

Fig. 3 Possible effects of dhCer on adipocytes. The ablation or inhibition of Des-1 by drugs such as Fen (4-HRP-fenretinide) in adipocytes leads to increased dhCer, (1) reducing adipogenesis and (2) increasing autophagy and resulting in increased insulin sensitivity and glucose uptake. (1) Increased dhCer reduces adipogenesis by (a) causing endoplasmic reticulum (ER) stress or nutrient stress which then phosphorylates eIF2 α downstream of PERK (Protein Kinase R-like Endoplasmic Reticulum Kinase), resulting in cell cycle arrest at G1, and (b) the increased dhCer also inhibits ligand activation of PPAR γ . Both of these lead to reduced differentiation of adipocytes due to reduced expression of cyclins D1, D3 and E and cdk2. (2)

DhCer also increases autophagy by reducing mitochondrial respiration and complex IV, which results in reduced ATP synthesis. The impaired ATP synthesis leads to increased AMPK, activating the phosphorylation of ULK1 (unc-51 like Autophagy Activating Kinase 1), Beclin 1 and LC3B II, which are involved in the initiation and formation of autophagosomes. This leads to increased expression of autophagy genes such as atg7 and E1-like, thus increasing autophagy. An increase in AMPK also increases GLUT4 translocation to the cell membrane, leading to increased glucose uptake. The hypothesis of dhCer acting as a ligand to activate RAR α thus inhibiting PPAR γ remains to be deciphered (light blue dotted line)

that are directed at determining whether dhCer has an associative or causal effect in CVDs.

DhCer in lung disease

Studies in lung diseases investigating dhCer were outnumbered by studies investigating Cer, S1P and Sph. As can be seen below, the few studies that did mention dhCer compared

its role in hypoxia as opposed to Cer. 4-HPR-fenretinide treatment of Sprague–Dawley (SD) rat lungs with emphysema showed that there was increase in the dhCer levels with a decrease in hypoxia-inducible factor1- α (HIF1- α) and vascular endothelial growth factor (VEGF) protein expression [54], which was rescued with concurrent S1P treatment. Additionally, it is now known that dhCer does accumulate in states of hypoxia through the induction of autophagy and

inhibits proliferation of primary rat lung-transformed cells [55]. These researchers proposed that the dhCer desaturation step acts as an oxygen sensor, based on the amplitude and kinetics of increased dhCer at physiological alterations of oxygen concentration. This can be explained by the requirement for oxygen by Des-1 and -2 to convert dhCer to Cer in the reaction involving nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NADH) [15]. In immortalized lung epithelial cells (IB3, A549 and C38) with defective expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, the levels of C16:0 dhCer, Sph, SM and Cer (C22, C24 and C26) were increased [52]. The use of 4-HPR-fenretinide and fumonisins (FB₁) reduced the level of these sphingolipids (individual species measurement not given) without affecting the level of CFTR, showing that CFTR could function in a feedback loop manner, sequestering sphingolipids and/or altering the membrane structure. The increase in dhCer in mice with defective CFTR gene expression is comparable to the increase seen in those with emphysema, since both pathologies have an underlying hypoxic condition. However, in states of infection, the response differs, as shown by the increased airway sensitivity caused by reduced levels of de novo sphingolipids including dhCer (due to deletion of SPLTC2) in mice lung infected with rhinovirus [104], showing that sphingolipids may be protective in lung hypersensitivity reactions. These studies show regulating dhCer levels by targeting the enzymes involved in its modulation could be potential therapeutic targets for hypoxia-related disorders in the lung. However, whether the increased dhCer contributes to the disease or occurs as a coping mechanism is yet to be deciphered.

DhCer in liver disease

The excessive accumulation of lipids within hepatocytes is one of the factors listed in the pathogenesis of fatty liver or non-alcoholic fatty liver disease (NAFLD), which can progress to hepatic fibrosis and cancer if not managed well. Raised dhCer levels together with Cer have been observed in both NAFLD and hepatocellular carcinoma patients when compared to hepatitis C infection and cirrhosis patients, respectively [58, 59, 105]. However in diabetic patients with NAFLD, up to 12% of increase in dhCer has been noted, with negative correlations with insulin resistance [106]. However, cell and animal studies show some conflicting results. For example, reductions in the de novo sphingolipid pathway (knockout of SPTLC1) led to the occurrence of fatty liver, insulin resistance and elevated fasting glucose in mice [107], while knockdown of Des-1 in Huh7 hepatocyte cells led to increased dhCer, FFAs and diacylglycerol [57]. This study also showed that silencing SPLTC1–3 showed positive effects such as increased nutrient uptake

and reduction in lipid synthesis, whereas Des-1 silencing led to prominent changes in amino acid, sugar, and nucleotide metabolism and vesicle trafficking between organelles in Huh7 hepatocyte cells. These contrasting effects may be reconciled if SPLTC2 and 3 are considered to be still functional in the former study. This also implies that the ablation of Des-1 in hepatocytes may be detrimental, since increasing levels of FFA and diacylglycerol can cause lipotoxicity which activates a chain of events that eventually leads to hepatocyte death.

In human hepatocarcinoma (HepG2) cells, interleukin 1 (IL-1)-mediated sterile inflammation downregulated orosomucoid like protein 3 (ORMDL3), a key regulator of SPT, leading to increased dhCers, dhSph and Cers [56]. Also, an integrated lipidomics and transcriptomics study in balb/c mice showed that the anti-inflammatory and immunosuppressive drug triptolide caused reductions in dhCer C18:0, C18:1, C20:0, C22:0 and C24:0 in the liver, and C22:0, C24:0, and C24:1 in plasma [108]. These studies suggest that inflammatory processes can also affect alterations in the level of individual species of dhCer in the liver and contribute to liver pathologies.

In the liver, dhCer together with sphingolipids seems to be part of the lipid pool that accumulates in disease states. However, due to the limited amount of studies specifically targeting dhCer in the liver, whether it has any effect remains to be answered.

DhCer in cancer and cancer therapy

As the investigation on Cer increased in cancer cells for combination therapy with various cancer treatments, due to its apoptotic property [109] it became apparent that dhCer could be bioactive. Most studies have regarded dhCer as a precursor to Cer [110–112]. However, there are studies that have demonstrated dhCer's potential role in cancer cell autophagy [14, 66, 113], in cancer induced bone pain [114] and cell cytotoxicity [115]. The changes in the levels of dhCer and Cer in cancer cells also seem to differ according to the site of origin of the cancer. For example, in melanoma cells, dhCers (d18:0/16:0) and Cers were significantly lowered compared to non-malignant melanocytes [116], while in cancerous tissue of human endometrial cells the level of dhCer was increased 3- to 4.6-fold, and Cer and S1P were increased 1.6- to 1.9-fold [117]. The most effective way to understand the effects of dhCer on a biological system is through the inhibition of the gatekeeper enzyme, Des-1, which is now a target for cancer therapy.

DhCer induced autophagy as a result of Des-1 inhibition

The Des-1 inhibitor, 4-HPR-fenretinide, is currently under clinical trial for use in breast cancer therapy [118]. The

anti-cancer effects of 4-HPR-fenretinide are thought to occur through the modulation of endogenous sphingolipids. A study by Rahmaniyan et al. [119] showed that 4-HPR-fenretinide does directly inhibit Des-1 with an IC_{50} of 2.32 μ M in SMS-KCNR neuroblastoma cells. Others have shown that inhibiting SK sensitizes cells to 4-HPR-fenretinide's cytotoxic effects due to increased dhCers [120]. These show that there is possible interaction between 4-HPR-fenretinide inhibition of Des-1 and SK activity, which has also been noted by others [113, 121–123]. Apart from these pharmacological agents, oxidative stress can also inhibit Des-1 in cancer cell lines such as HEK293, MCF 7, 549 and SMS-KCNR cells, leading to increased dhCers [124]. The raised exogenous dhCer levels seems to be capable of inducing autophagy; as shown in T98G, U87MG glioblastoma cells [66] and DU145 cells [14] and also reduce the proliferation of castration-resistant prostate cancer cells [125]. In the prostate cancer cells, reduction in proliferation occurred without inducing apoptosis and autophagy, perhaps through effects on the cell cycle. Additional support for dhCers autophagic effects in cancer cells is found in a study on human gastric cancer cell line, HGC-27, where the inhibition of Des-1 by XM462 and resveratrol led to the accumulation of dhCer at 16 h with induction of autophagy, whereas Cer was increased only slightly [113]. In addition, another study on U937 cells showed that dhCer did not induce apoptosis through DNA fragmentation, compared to Cers and tumour necrosis factor- α (TNF- α) [126]. The autophagy effect of dhCer seems to occur only when the de novo sphingolipid biosynthesis pathway is altered. This is because in studies where dhCer levels increased together with Cers, apoptosis occurred rather than autophagy. For instance, the anti-tumour effect of TNF- α in MCF-7 cells occurred through increased activity of CerS, which then drove the de novo sphingolipid synthesis forward, leading to accumulation of dhCers (C16:0, C18:0, C20:0, C22:0, C24:0, C24:1) and Cers [127] and thus regulating focal adhesion kinase (FAK) and apoptosis. Since the role of autophagy in tumours is highly context driven and can lead to either regression or advancement of tumours [128], this could also apply to targeting Des-1 inhibition as an anti-cancer therapy. This is evident in a recent study in leukaemia cells which found that dhCer accumulation and ROS generation were distinct and non-essential events in 4-HPR-fenretinide-induced cell death [129]. This is further confounded considering that 4-HPR-fenretinide can have both retinoic acid (RA)-dependent and -independent effects [34], and that it induces polyubiquitination of the enzyme [83]. Apart from these, Des-1 inhibition is also promising in terms of restraining metastasis. Studies have linked Des-1 to promotion of metastasis in prostate cancer cells [130], and oesophageal carcinoma [131]. It is worth mentioning that this promotional effect was regulated by RA without affecting the proliferative potential of the

cell [130], maybe because Des-1 also increases cyclin D1 expression as a result of NF- κ B activation [131].

In an effort to beat resistance to Foscan photodynamic therapy (PDT), some have studied its combination with 4-HPR-fenretinide. Their findings showed that the apoptotic effect was greater when combined, compared to either alone in SCC19 cell by increasing dhCer C16:0 and not Cer [132]. This combination also enhanced mitochondrial depolarization. PDT alone has been shown to induce accumulation of dhCer in SCC cells [133, 134] and was thought to effect the resistance by inhibiting the formation of ceramide channels in the mitochondria [133]. The reason for the enhanced effect when combined may be due to enhanced CerS activity and mitochondrial dysfunction [132]. This is supported by two different studies by Separovic et al. [135], which showed that SCC cells with silenced CerS1 or knockout of CerS6 genes treated with PDT had reduced levels of global Cers, dhCers (C18:0, C18:1 and C20:0) and decreased apoptosis. These findings also imply ROS as a mediator between Des-1 and CerS, since PDT induces cell cytotoxicity through ROS generation.

These studies contribute to the evidence that raised dhCer levels could potentially mean increased autophagic flux. Collectively, increasing dhCer levels to increase autophagy and inhibiting metastasis through Des-1 inhibition are promising targets for cancer therapy.

DhCer induced ER stress

Vitamin E, γ -tocotrienol (γ -TE), has been demonstrated to confer its anti-cancer effects through modulation of dhCer. A study by Jiang et al. [136] showed that γ -TE induced autophagy, necrosis and apoptosis in prostate cancer cells by increasing intracellular dhCer and dhSph, suppressing Akt phosphorylation. Suppression of the PI3K/Akt signaling pathway which leads to inhibition of NF- κ B is a known target for γ -TEs anti-breast cancer effects [137]. In fact, in RAW264.7 macrophages, the shorter chain dhCer, C8:0, was linked to the anti-NF- κ B effects of γ -TE, by enhancing ER stress and attenuating TNF- α -triggered increase in NF- κ B [138]. What is interesting to note in this study is that dhCer C8:0 mimicked the effects of γ -TE by increasing the expression of the zinc finger protein A20, which is a negative feedback regulator of NF- κ B. This also led to increased phosphorylation of eIF2 α , cJun N-terminal kinase (JNK) and NF- κ B inhibitor α (I κ B α). Phosphorylation of the ER stress marker, eIF2 α , has also been noted in adipocytes treated with 4-HPR-fenretinide [34]. In contrast, increased A20 in adipocytes has been shown to enhance adipogenesis by suppressing NF- κ B even in the presence of TNF- α [139]. These differences may be due to different NF- κ B pathways being activated: canonical (involves TNF- α) vs. non canonical, apart from cellular differences. It is also possible that

γ -TE may be inhibiting Des-1 or even the expression of certain CerS. Interference of the expression of CerS2, 5 and 6 has been shown to increase dhCer C16:0 and hexosylceramide which also promoted ER stress [140]. This study also noted that the observation of the expression levels of individual CerS in MCF-7 cells leads to counter regulation of non-targeted CerS species with no significant differences in total sphingolipids.

DhCer in other diseases

In the kidney, Cer triggers the mitogen-activated protein kinase (MAPK/ ERK) cascade in glomerular mesangial cells [141] and the stress-activated protein kinase (SAPK/ JNK) cascade in the endothelial cell; however, dhCer was not able to trigger the SAPK/JNK cascade [142] and whether it triggers the MAPK/ERK is yet to be deciphered. Dermatological studies have indicated dhCer's possible role in heterogeneity of the stratum corneum layer [143]. In addition, others have found altered expressions of the enzymes CerS, cDase and SPT in the skin disease, "hidradenitis suppurativa" [144]. However, lack of measurement of the different sphingolipids was a limitation in this study. In the eye, increased dhCer (C18:1, C16:0) has been indicated as a possible contributor to cataracts in 64–70 year old [145].

Collectively, dhCers' association with hypoxia possibly triggering autophagy is a recurrent finding in the brain, diabetes, aging, lung and cancer. The relevance of this effect depends on the pathophysiology of the disease, therefore

indicating its potential applications as a biomarker or therapeutic target. The mechanistic aspects of this link between dhCer and hypoxia remain to be elucidated. Figure 4 gives a summary of the possible effects of increased dhCer as highlighted in this review.

DhS1P in disease

Overview and structure

DhS1P is derived from the phosphorylation of dhSph by SK1 and 2, and it is known to accumulate when CerS is inhibited [146]. It differs from S1P in that its backbone structure is composed of dhSph instead of Sph. The role of its chemical analog, S1P, as a signalling molecule in the regulation of cellular processes such as cell proliferation [147, 148] and neuroprotection [149] are now known and are being targeted for therapy. As in the case of the other dihydropshingolipids, research into the relevance of dhS1P in the cellular mechanisms of disease is fairly new and quite limited.

DhS1P in cerebrovascular disease

DhS1P has been shown to activate S1PRs [150] in neuronal progenitor cells, and the orphaned receptor GPR63 in the thalamus and nuclear-caudatus of the brain [151]. Recent studies have demonstrated reduced dhS1P levels in the

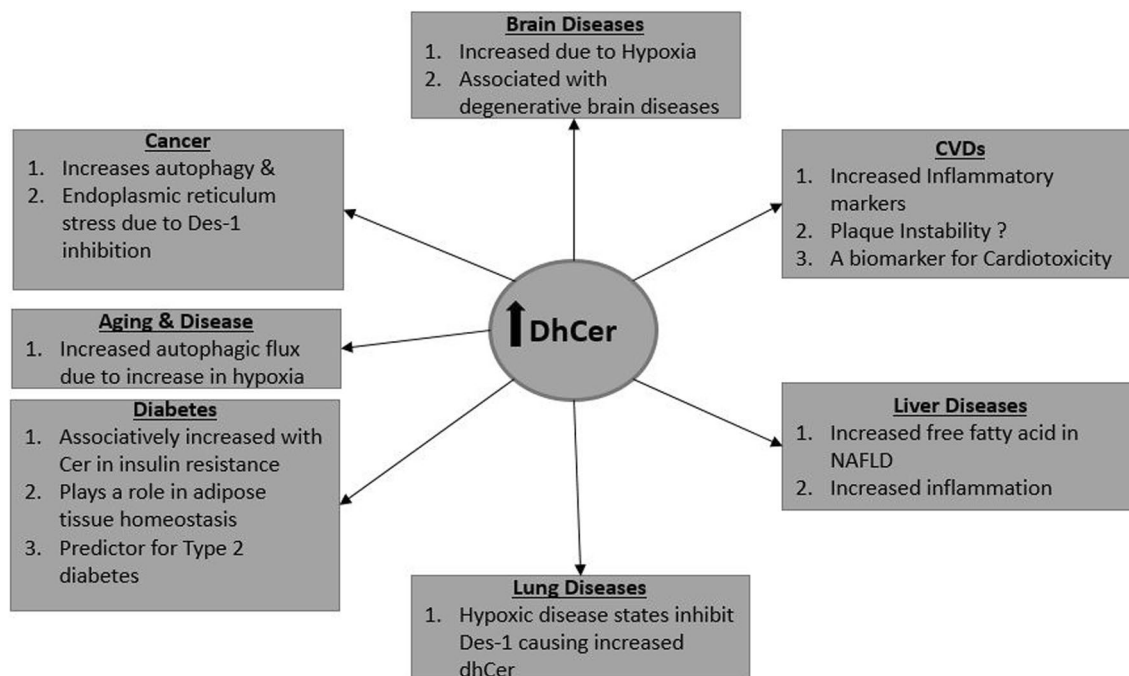


Fig. 4 DhCer in diseases. A summary of the potential effects of increased dhCer as highlighted in this review

brains of rat models of Alzheimer's disease [152] and HD [62]. The reduced availability of dhSph due to a perturbation in the de novo synthesis pathway may lead to reduced dhS1P levels, since it occurred in conjunction with reduced levels of dhSph and dhCer and the enzymes SPTLC1 and CerS1. Raised DhS1P may have a protective role in HD, since the accumulation of nuclear dhS1P has been shown to inhibit histone deacetylases (HDAC) [146], which is being targeted for HD therapy [153, 154]. The inhibition of HDAC results in increased gene expression that leads to increased cell proliferation, migration and decreased cell apoptosis. In addition, studies in neuronal cells also found that dhS1P increases Smad phosphorylation compared to S1P [150]. Smads are involved in neuronal precursor proliferation and differentiation. However, in nerve cells (PC12), dhS1P did not protect the cells from apoptosis, whereas S1P did [155]. The different cellular microenvironments could be the reason for this difference. This is exemplified by the inhibition of TGF β -induced Smad 2/3 phosphorylation by dhS1P in dermal fibroblasts [156], which is opposite to the effects seen in neuronal cells. Other studies have also shown that the pharmacological inhibition of Des-1 in cerebellar neuron cells [157], hypoxia in cerebellar endothelial cells [23], and CerS inhibition by FB1 in neuronal progenitor cells [158] can raise the dhS1P levels. In addition, dhS1P has been identified as a potential marker in FB1–neural tube defect risk assessment [158]. These studies show that the inhibition of Des-1 or CerS reverses the sphingolipid metabolism reaction towards the dihydroshingolipids and that of dhS1P, possibly by interfering with the activity levels of SPT and S1P lyase. It is obvious that DhS1P does have some form of influence on neuronal cells proliferation and differentiation, and could be a potential therapeutic target for neurodegenerative diseases such as HD.

DhS1P in cardiovascular disease

Similar to S1P, plasma erythrocyte and platelet levels of dhS1P differ in physiological states. In states of physical strain such as exercise, the dhS1P levels differ according to the type of activity, duration and training [159–161]. For example, in untrained man, the erythrocyte levels of dhS1P at 60 min of pedalling were elevated and remained markedly elevated post-exercise [159]. Thus, these differences are also most likely to be present in pathophysiological states.

Both in animal models of cardiomyopathies and patients with cardiomyopathies, altered sphingolipid levels have been noted. Having a major cardiac event such as a myocardial infarct (MI) has been shown to alter the levels of dhS1P in plasma (reduced at 1–6 h), erythrocytes (increased at 6 and 24 h), and platelets (reduced) in rats [162]. Reduced dhS1P and S1P have also been observed in left ventricular tissue of Wistar rats subjected to tachycardia [163]. Similar trends in

plasma (reduced early on) and erythrocytes (increased early on) have been observed in patients with acute ST-segment elevation myocardial infarct (STEMI) [164], and MI [165]. It has been suggested that reduced plasma S1P enables erythrocytes to increase S1P production by increasing SK1 protein expression and activity [166]. Hypothetically, this may also be the case for dhS1P, since both were incidentally increased or decreased. Samples from patients in the Copenhagen City Heart Study (CCHS) showed that there was an inverse relationship between reduced dhS1P, S1P and Cer C24:1, and the occurrence of ischaemic heart disease (IHD) in the plasma fraction containing HDL [167]. This may be due to the decreased availability of HDL, implying that dhS1P may be bound to HDL just as S1P [168]. S1P is known to be positively and negatively correlated to CAD depending on the plasma HDL or non-HDL fraction it is bound to [70], while S1P released from activated platelets preferentially binds to the non-HDL fraction—Albumin [169]. Studies that have investigated dhS1P together with S1P have shown that dhS1P is found in non-activated platelets [170, 171], and it was increased in and released by activated platelets [170, 172]. Whether albumin-bound dhS1P and S1P influenced the outcomes observed in the CCHS study was not investigated.

Moreover, a shift in the balance between dhS1P, S1P and Cer within the platelets rather than erythrocytes may be aiding the cross talk in CAD, as observed in patients with multi-vessel CAD [171]. Together with the findings of reduced dhS1P contributing to reduced endothelial barrier [173], its positive correlation with increased miRNA-122 and 126 in improved endothelial barrier function [174], and dhS1P as a potent inducer of S1PR1-dependent endothelial barrier function and endothelial cell migration [167], it can be inferred that dhS1P may promote plaque stability. Even in human umbilical vein endothelial cells (HUVEC), dhS1P has been shown to inhibit chemotaxis and Rac activation stimulated by platelet-derived growth factor (PDGF) [175], which is known to promote atherosclerosis. Furthermore, dhS1P has been shown to induce matrix metalloproteinase 1 (MMP1) in dermal and scleroderma fibroblasts [176, 177], which is involved in plaque stability [178] and linked to reduced risk of coronary heart disease [179]. The downregulation of MMP1 is also a known marker for cardiac fibrosis. The study on scleroderma fibroblasts showed that dhS1P not only normalized MMP1 expression through the upregulation of phosphatase and tensin homolog (PTEN), but also inhibited factors known to promote fibrosis such as phosphorylated Smad3 (pSmad3), and collagen [177]. In the dermal fibroblasts, dhS1P induced the ERK 1/2-Ets1 pathway, leading to increased MMP1 through one of its pertussis toxin-dependent receptors. In the setting of atherosclerosis, this pathway facilitates and promotes vascular smooth muscle cell proliferation, thus promoting fibrous cap stability, while S1P led

to the induction of the inflammatory factor, cyclooxygenase 2 (COX-2) in the same study. However, MMP1 activation or reduction by dhS1P in endothelial cells is not known, It is possible to hypothesize from these studies that dhS1P may also play a role in vascular fibrosis. Considering the common factors involved in fibrosis in the cardiac and circulatory system such as the renin–angiotensin–aldosterone system (RAAS), the effects of dhS1P in the cardiac system needs to be investigated. Furthermore, raised dhS1P levels were demonstrated to have a strong relationship with survival from cardiac arrest in SK1-knockout mice, while S1P did not [180]. The increase in dhS1P can be attributed to the increased activity of the enzyme SK2, which is localized in the nucleus [181]. Taking into account the inhibitory effects of dhS1P on HDAC in the nucleus [146], the potential for it to impact on survival through increased proliferation is highly likely. Another area that warrants further research is studies detailing what impact commonly prescribed cardiac medications may have on dhS1P's role in CVDs, since dhS1P levels were shown to be reduced in plasma of healthy subjects taking a 300 mg loading dose of aspirin [182].

These animal and clinical studies clearly show that dhS1P may be involved in the pathophysiology of CVDs and that platelets and erythrocyte levels of dhS1P influence the plasma levels of dhS1P and S1P for that matter. Applying this to CAD, hypothetically, there could be increased albumin-bound dhS1P. However, how this may influence the outcome of the disease is unknown, especially since the studies also show that dhS1P may promote plaque stability through improved endothelial barrier function. Another area that warrants further research is dhS1P's role in cardiac fibrosis.

DhS1P in lung disease

In terms of lung diseases, sphingolipids and sphingolipid metabolism have been suggested as potential contributors to the pathogenesis of asthma [183], especially in relation to the interactions between ORMDL3 and SPT. A recent study has shown that the inhibition of ORMDL3 increased SPTLC1 and S1P, which then increased smooth muscle contraction rather than inflammation, causing airway hypersensitivity (AHR) [184]. Increases in both S1P and dhS1P have been noted in relation to dust mite allergy, increasing AHR and the asthmatic phenotype [185]. However, it is likely that the prominent increase in S1P led to the effects. The immunomodulatory molecule FTY720, which is known to reduce ORMDL3 leading to reduced AHR and inflammation [186], was able to inhibit CerS4 and increase SK1, leading to decreased S1P and increased dhS1P levels in human lung endothelial cells [53]. This suggests that therapeutic agents such as FTY720 could be more useful than those that inhibit ORMDL3 alone, assuming dhS1P potentially has a different

effect than S1P. Furthermore, Berdyshev et al. [187] have shown in their study that the increase in SK1 derails the metabolic pathway of sphingolipids towards that of dhS1P generation, rather than S1P in respiratory syncytial virus (RSV) infection of human bronchial epithelial cells (HBEpC) and HPAEC. They also suggested that SK1 forms a substrate membrane enzymatic complex that impacts on this derailment. Additionally, dhS1P has been shown to compete for cystic fibrosis transmembrane receptor uptake with S1P in C127 cells [188], while in the setting of radiation-induced pulmonary fibrosis both S1P and dhS1P, and the expression of SK1 were increased [189]. Considering the contrasting findings in dermal cells and neuronal cells in terms of dhS1P in activating or inhibiting certain fibrotic factors, and those of S1P in cardiac fibrosis, the role of dhS1P in pulmonary fibrosis needs to be investigated. What is apparent in these latter studies is the regulation of dhS1P and S1P by SK1 increase may be stimulus, cell type, and complex dependent as hinted by Berdyshev et al. [187].

DhS1P in liver and kidney disease

Studies have demonstrated the protective effects of dhS1P against ischaemic–reperfusion injury (IRI) in mice hepatic and renal tissues [190, 191]. DhS1P was able to confer protection against IRI by activating S1PR1, which led to phosphorylation of MAPK/ERK, Akt, and heat shock protein 27 (HSP27) [190]. Exogenous treatment of the mice subjected to hepatic IRI with low doses of dhS1P led to reduced hepatic and renal necrosis and apoptosis, neutrophil infiltration, preserved endothelial cell integrity and reduced pro-inflammatory mRNA [191]. It should be noted that there were no changes observed in S1P levels and S1P conferred protection through S1PR3. DhS1P has also been recommended as a marker for FB1 toxicity [192]. This is supported by studies in cells [193], ducks [194] and human [195] serum or tissue, which showed an increase in dhS1P after exposure to FB1. Apart from it being a marker for toxicity, it may also contribute to cell proliferation. The accumulation of dhS1P due to FB1 toxicity in renal cells led to transient activation of PKC α within 5 min of exposure, compared to dhSph, Sph, S1P and Cer [193]. PKC α mediates the mitogenic effect of PDGF in renal mesangial cells (RMC) [196]. PDGF has been shown to induce increased expression of SK1 mRNA [197], which diverts dhSph towards phosphorylation to give dhS1P instead of Cer, promoting cell survival [198]. In addition, both S1P and dhS1P were able to stimulate similar gene expression waves as PDGF in RMC [199]. In dhS1P-stimulated cells, the angiotensin II receptor type 2 (AT2R) expression was lower than in S1P-stimulated cells, implying that dhS1P has a higher mitogenic effect. In fact, this study also showed that dhS1P had a greater degree of intracellular calcium mobilization than S1P, which explains the transient

activation of PKC α seen in FB1 toxicity [193]. The calcium/PKC pathway is one of the signal transduction pathway for growth factors such as PDGF. Both dhS1P and S1P also induced growth factors such as heparin-binding EGF-like growth factor (HB-EGF) and connective tissue growth factor (CTGF), a fibrotic protein, which was not induced upon stimulation with PDGF [199].

It can be summarized from these studies that dhS1P is able to activate proliferation either on its own through the calcium/PKC pathway or by interacting with other signalling molecules, including ERK, MAPK and Akt, and HSP27 in the kidney and liver at lower doses while conferring toxic effects at higher doses. How this may impact upon the long-term systemic effects such as fibrosis and in the setting of different pathologies needs to be evaluated.

DhS1P in cancer and cancer therapy

In terms of cancer therapy, dhS1P may help promote survival of neuronal cells [200], inhibit migration, invasion of melanomas through S1PR2 activation [201] and could even be harnessed as a therapeutic tool for tumours [202]. In C6 glioma cells, dhS1P was able to activate the ERK/early growth factor response 1 (EGR-1)/fibroblast growth factor 2 (FGF-2) pathway through S1PR1 [200]. FGF-2 is a neurotrophic factor involved in neuronal differentiation and survival. DhS1P also activated phospholipase D (PLD), a mitogenic factor, through S1PR2 but at lower levels than S1P. The activation of S1PR2 by dhS1P and S1P in B16 melanoma cells led to inhibition of cell migration through regulation of RhoA and Rac which are involved in cell motility [201]. One of the limitations to cancer treatment has been the systemic immune suppression caused by tumour-associated inflammation effected through myeloid lineage cells. Barth and colleagues showed that a recent therapeutic tool targeted at this phenomenon, termed “Photo-ImmunoNano-Therapy”, improved the outcome in mice models as a result of dhS1P (S1P to a lesser degree) abrogating myeloid lineage cells and allowing the expansion of anti-tumour lymphocytes [202]. The increase in dhS1P was attributed to increase in SK2, which is known to have epigenetic effects [203], rather than SK1. They also injected tumour-bearing mice with dhS1P and found it to have anti-tumour effect, while S1P promoted tumour growth. Incubation of T cells stimulated with the immune-suppressive drugs anti-CD3 and anti-CD28 with dhS1P induced the release of interleukin 2 (IL-2) and interferon- γ (INF- γ), respectively [204]. Thus, dhS1P inhibits T cell proliferation which could suppress tumour growth and survival. However, this may not be true for all types of cancers, since patients with hepatocellular carcinoma were found to have raised serum dhS1P levels. Despite this, it can be surmised that dhS1P is a potential anti-cancer biomolecule that needs to be further investigated (Fig. 5).

DhSph in disease

Overview and structure

Sphinganine or dihydrosphingosine (dhSph) forms the backbone of dihydrosphingolipids. It has a molecular weight of 301.5 g/mol and is produced mostly in the endoplasmic reticulum. DhSph serves as a precursor to dhS1P synthesis by SK1 and 2 and dhCer by ceramide synthases. In biological systems, early studies in the 1990s seem to have used dhSph as a protein kinase C (PKC) inhibitor with regard to cell proliferation and vasoconstriction studies [205–207]. Here, we look at its role in different diseases.

DhSph in hepatic and renal diseases

Much relevance has been given to the enzymes involved in dhSph metabolism, thus overlooking its role in pathophysiology. Only a few studies have considered dhSph, especially in terms of FB1 toxicity which increases the dhSph and dhSph/Sph ratio. The extent of FB1 toxicity in humans has been reviewed by Voss et al. [208]. A number of studies have found raised dhSph levels due to FB1 exposure in the liver and kidney [209], the brain of calves [210], gastrointestinal tract (GIT) of chickens [211], pregnant mice and fish (with no fetal toxicity) [212, 213], and in urine samples from humans [214]. Apart from FB1 toxicity, dhSph was also increased in the plasma in other instances, such as in hepatotoxicity due to *Guynuria Segetum*, Fabry’s disease, endemic nephropathy, hepatitis C infection, type 2 diabetes-induced NAFLD, disease models of glucocorticoid-induced osteoporotic rats and dyslipidaemia, remote ischaemic preconditioning (RIPC) strategy for IRI, and genetic ablation of CerS2 in the liver [106, 215–222]. The key factor in all of these increases is the inhibition of the CerS enzyme which catalyses the acylation of dhSph to dhCer. FB1 competitively inhibits CerS due to it being structurally similar to dhSph and differing only in the free amino group at C₁ [223]. This inhibition not only raises dhSph levels, but also the levels of dhS1P which is known to have autocrine–paracrine functions on the S1PRs, further complicating the mechanistic pathways of FB1 toxicity. Whether or not the increased dhS1P is also able to inhibit CerS2 by directly interacting with the S1P receptor-like motif on CerS2 is unknown [224]. FB1 toxicity is accompanied by an increase in TNF- α expression causing increased cell apoptosis and induction of cytokines such as IL-12 p40 and IFN γ [225, 226]. However, He et al. [227] stated that this is not directly related to the increase in dhSph or Sph as shown by the continuous

expression of TNF- α despite the inhibition of SPT in the presence of FB1 in kidney cells. However, earlier studies by Sharma et al. [228] in TNF- α receptor knockout mice showed that there was some increase in dhSph in the liver and kidney, but these were lower than in the wild types. These studies imply that there may be partial interactions between TNF- α and dhSph or the de novo pathway.

Reduced dhSph levels have been noted in the seminal plasma of infertile male patients with Kidney–Yang syndrome [229], adenine-induced chronic renal failure in rats [230], and in type 2 diabetes-induced diabetic nephropathy [231]. These studies were metabolomics and metabonomics studies aimed at discovering biomarkers for these disease conditions. Their findings showed the sphingolipid metabolism may be perturbed, the mechanisms of which remain unknown. Regardless, it is likely that the beginning of the de novo pathway is perturbed in these disease conditions, causing the reduced levels seen. DhSph has also been mentioned as a possible biomarker for kidney cancer [232]. It is worth considering the causal increase in dhSIP levels in these studies which could influence the outcomes observed. Therefore, to explore the effect of dhSph, research that takes into account this aspect would be valuable.

DhSph in cardiovascular disease

Elevated levels of dhSph have been noted in the hearts of rats exercising to exhaustion in 30 min [233], or pacing for 60 min [234], both of which show that increased cardiac workload not only affects SLs levels, but dhSLs as well.

Cardiomyopathies

In terms of cardiomyopathies, various other researchers have shown altered dhSph levels. For example, raised levels of dhSph were shown in plasma and tissues from rat MI models [162, 235–237], in the right ventricle after 60 min of tachycardia [163] and in cardiac muscle of male Wistar rats with drug-induced hyperthyroidism [238]. DhSph and phytosphingosine were identified as biomarkers in relation to the efficacy of traditional Chinese medicine (TCM) therapies in two of the MI studies [236, 237]. Phytosphingosine is derived from dhSph (as characterized in yeast) and causes apoptosis of cancer cells by caspase 8 activation and Bcl-2-associated X protein (Bax) translocation [239]. However, another study employing similar analytical methods and experimental conditions for MI indicated phytosphingosine as a biomarker and not dhSph [240]. The reason for this may lie in the rate of metabolism of dhSph in the tissue and plasma. The latter study was carried out on heart tissue. Reducing solid tissue de novo synthesis of sphingolipids were also shown to affect the level of dhSph in plasma (decreased) and platelets (increased) [241]. The

disruption of the sphingolipid metabolic pathway showing increases in dhSph in cardiomyopathies has also been shown in the plasma of young (STEMI) patients [164, 242]. This study showed that dhSph had high specificity and sensitivity to the prognosis related to major adverse cardiovascular events after patients were discharged [242]. Prior to this study, 25–27% reductions in plasma dhSph were reported in chronic systolic heart failure patients, independent of the underlying cause of heart failure [243], with no changes observed in the plasma level of S1P and dhS1P, perhaps due to metabolic clearance as noted in another study where urine levels of dhSph and phytosphingosine were increased in HF patients [244]. Disease onset and duration could have also influenced these findings. For example, the STEMI study reported elevated levels upon admission, which were reduced at 1, 5, and 30 days after admission, while others have shown no changes in plasma dhSph levels in MI patients at the time of admission and 5 days after [165].

Coronary artery disease

Raised dhSph levels have been indicated in the progression of atherosclerotic dyslipidaemia [245], in spontaneously hypertensive rats [246], and has also been investigated as a biomarker for atherosclerosis in a rabbit model [247]. In patients with multi-vessel CAD, the level of dhSph and Sph in platelets has been shown to be higher than in the controls, whereas their levels in plasma and erythrocytes were stable or similar [171]. In addition, a study in patients with temporary coronary occlusion found that 1 min after PCI in the coronary sinus, dhSph levels were raised to 614%, and 272% in peripheral blood, but dropped below baseline at 12 h [248]. The inhibition of SPT by myriocin in apolipoprotein E (ApoE)-deficient mice led to significant reductions in dhSph and other sphingolipids levels, with a stable plaque formation and reductions in cholesterol and LDL [249], but in ApoE null mice fed with a high fat diet, dhSph levels were raised which positively correlated with total cholesterol and LDL-C [245]. Thus, inhibition of the sphingolipid de novo synthesis pathway may be beneficial to lowering atherogenic plasma lipids and encourage stable plaque formation. However, studies that could inform the mechanisms of this interaction between cholesterol and dhSph or sphingolipids are lacking. Therefore, these findings are speculative at this time.

DhSph in other diseases

The intracellular increase in dhSph is either as a result of overall increase in the de novo sphingolipid synthesis leading to effects similar to that of Cer, or due to inhibitions at the CerS enzymes, the effects of which are still elusive. The extracellular addition of dhSph also leads to Cer-type effects

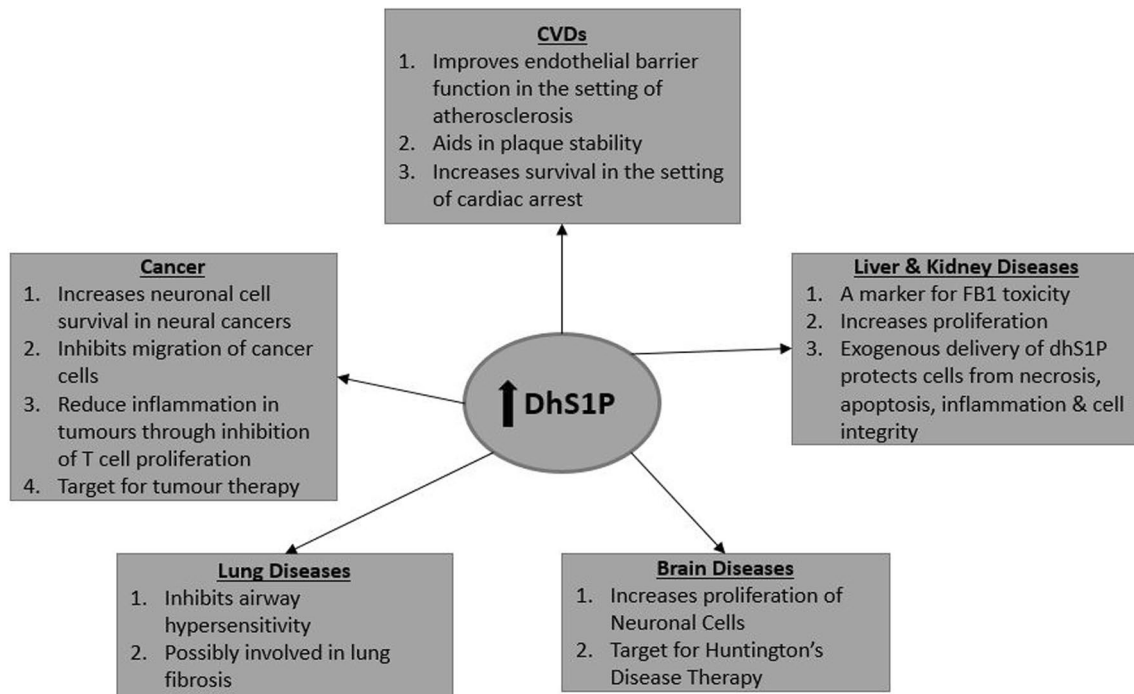


Fig. 5 DhS1P in disease. A summary of the potential effects of increased dhS1P as highlighted in this review

Table 2 List of sporadic studies on different disease models of SPT or CerS interventions with effects on dhSph levels

Disease or disease event studied	Target organ/tissue	Experimental model	Method of de novo pathway perturbation	Changes in dhSph	References
Hypoxic state	Brain	Human cerebral endothelial cells	CerS inhibition	Increase	[23]
Colitis	GIT	Mice	CerS2 knockout mice	Increase	[259]
Gastric smooth muscle dysfunction	GIT	Mice	CerS2 null mice	Increase	[260]
Pancreatitis	Pancreas	Human plasma	De novo synthesis pathway ^a	Increase	[261]
Myopia	Eye	Human aqueous humour	De novo synthesis pathway ^a	Increase	[262]
Rheumatoid arthritis	Joints	Human Plasma	De novo synthesis pathway ^a	Increase	[263]
Pre-eclampsia	Uterus	Human plasma/placenta	De novo synthesis pathway ^a	Increase	[264]
Rhino virus infection	Lungs	Rat	Deletion of SPLTC2	Decrease	[104]
Wolfram syndrome	Brain	Human plasma	De novo synthesis pathway ^a	Decrease (C17:0)	[265]

^aMetabolomics or metabonomics studies that show the de novo synthesis pathway may be perturbed, indicating dhSph as a biomarker. However, the mechanisms of this perturbation are less understood

such as apoptosis in cancer cells [250]. De novo sphingolipid synthesis can be perturbed by inhibiting or overexpressing the enzyme SPT. The yeast orthologues of ORMDLs have been shown to inhibit SPT by forming a conserved complex with SPT reducing sphingolipids such as dhSph [251, 252]. Lowering the level of the enzymes at both ends of the de novo pathway such as that seen HD rat models [62]: SPLTC1 and CerS1, results in reductions in dhSph, dhCer and dhS1P. However, dhSph could be a promising target for therapy in dermatological diseases such as atopic dermatitis, where Sph

and dhSph ratios were found to influence barrier abnormalities observed in human stratum corneum (SC) [253]. For example, dhSph was found to play a role in contributing to the formation of more rigid lattice of lipids in the SC [254]. It has also been suggested as a biomarker in neurodegenerative disease and diabetes. The altering of dhSph levels in diabetic disease states and models by the inhibition of the sphingolipid pathway or anti-diabetics that regulate lipid and cholesterol also supports dhSph being a possible biomarker for diabetes and diabetes therapy [255–258]. Such

applications could allow for early detection of insulin resistance and patient response to therapy, because it is a necessary step in the de novo pathway that leads to Cers. Overall, studies in which dhSph is implicated are sporadic, which makes them difficult to discuss; therefore we have collated them in Table 2.

3-Ketosphinganine in disease

3-KetoSph is the product of the condensation of palmitoyl-CoA and serine catalysed by the enzyme SPT in the ER, which is the rate-limiting enzyme in the de novo sphingolipid metabolism pathway. The inhibition of the enzyme SPT in relation to disease seems to be studied more than the effects of the product 3-ketoSph, due to it being metabolized rapidly. In fact, an increasing number of studies are reporting links of mutations in the gene that encodes SPT, SPLTC1 and 2, to hereditary peripheral neuropathies [266–268]. There are also reports of new novel SPT inhibitors for cancer that have shown to reduce 3-ketoSph in human lung adenocarcinoma cells [269]. Mutations or missense in the enzyme that reduces 3-ketoSph, 3-ketodihydrosphingosine reductase (KDSR), have been linked to recessive progressive symmetric erythrokeratoderma [270], keratinization disorders associated with thrombocytopaenia [271] and bovine spinal muscular atrophy [272]. Long-term exposure of cancer cells (HGC27, T98G and U87MG) to 3 ketoSph has been shown to induce autophagy and overexpression of Des-1 [273].

The evidence for 3 ketoSph in disease is quite scarce owing to its rapid metabolism in the de novo sphingolipid synthesis pathway; however, the enzymes involved in its synthesis and metabolism are targets for further studies.

Conclusion and perspectives

Collectively, the evidence for dihydrosphingolipids in disease is spatial across the board and thus requires a lot more research in terms of their roles in disease, especially the mechanistic pathways through which they could contribute to disease. There are a number of areas that have been examined in this review that should be the focus of further research. These include: (1) the value of dhCers in predicting type 2 diabetes in relation to obesity, (2) the possible role of dhCer in reducing adipogenesis and increasing autophagy in adipocytes, (3) the reoccurring theme of dhCer in association with hypoxia, (4) the role of dhS1P and dhSph in plaque stability, (5) the anti-tumour effects of dhS1P conferred through suppression of T cell proliferation, (6) the binding of dhS1P to albumin and the effects of this in terms of IHD, (7) the possible therapeutic effect of dhS1P in terms of HD, and (8) the stimulus, cell type and complex-dependent regulation

of dhS1P by SK1. There are also a number of studies in terms of CVDs showing alterations in sphingolipid levels; however, what is lacking are mechanistic studies to show if these alterations can contribute to the pathophysiology of the disease. The role of dhSph as a biomarker in cardiomyopathies, drug-induced toxicities, as well as liver and kidney toxicity due to FB1 is imperative, especially in determining if the de novo sphingolipid synthesis pathway is perturbed. Future studies applying current lipidomics tools should be encouraged, together with studies that take into consideration both the metabolites and the enzymatic interactions of the de novo pathway. The use of more potent and selective Des-1 inhibitors should be encouraged for investigating the effects of dhCer or Des-1 inhibition in light of the recent polyubiquitination findings for 4-HRP-fenretinide. Finally, the altering role of dihydrosphingolipids in the different organs seems to depend not only upon the initial insults and the disease processes, but also the key players along the de novo sphingolipid pathway.

Author contributions RM and BW conceived and designed review question, conducted preliminary data search, and sorting the papers. FS and YH conducted literature search and screening. DK, CR, BF and DL assisted drafting and edited of the paper and had responsibility for its final content. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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