Ceramides and mitochondrial fatty acid oxidation in obesity

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ABBREVIATIONS

ACC, acetyl-CoA carboxylase; AMPK, AMP-dependent protein kinase; CerS, ceramide synthase; CPT1, carnitine palmitoyltransferase; DES, dihydroceramide desaturase; DG, diacylglycerol; ER, endoplasmic reticulum; ETC, electron transport chain; FA, fatty acid; FFA, free fatty acid; FAO, fatty acid oxidation; IL-1β, interleukin-1β; IL-6, interleukin-6; KO, knockout; MCD, malonyl-CoA decarboxylase; SM, sphingomyelin; SPL, sphingolipid; SPT, serine palmitoyltransferase; TG, triglyceride; TNFα, tumor necrosis factor alpha; T2DM, type 2 diabetes mellitus; WAT, white adipose tissue; WHO, World Health Organization

ABSTRACT

Obesity is an epidemic, complex disease that is characterized by a state of increased glucose, lipids and low-grade inflammation in circulation, among other factors. This is the perfect scenario for the production of ceramide, the building block of the sphingolipid family of lipids, which is involved in metabolic disorders such as obesity, diabetes and cardiovascular disease. In addition, obesity causes a decrease in fatty acid oxidation, which contributes to lipid accumulation within the cells, conferring more susceptibility to cell dysfunction. C16:0 ceramide, a specific ceramide species, has been identified recently as the principal mediator of obesity-derived insulin resistance, impaired fatty acid oxidation and hepatic steatosis. In this review, we aim to cover the importance of ceramide species and their metabolism, the main ceramide signaling pathways in obesity, and the link between C16:0 ceramide, fatty acid oxidation and obesity.

Keywords

C16:0 ceramide; carnitine palmitoyltransferase 1; energy expenditure; insulin resistance

INTRODUCTION

"Globesity" is the word that the World Health Organization (WHO) uses to refer to the global epidemic of overweight and obesity, which is currently a major public health problem in many parts of the world. Obesity is no longer a problem of high-income, developed countries. Indeed, the largest increases in obesity since 1980 have occurred in low and middle-income countries, particularly in urban settings in Oceania, Latin America, and North Africa (1).

Obesity reflects an imbalance between energy intake and energy expenditure and is characterized by excessive fat accumulation in adipose tissue and other organs that has a negative impact on health. It has been established that obesity is a risk factor for other pathological conditions such as insulin resistance and type 2 diabetes mellitus (T2DM) (2), as well as non-alcoholic fatty liver disease (3), cardiovascular disease (5) and cancer (6), among others. During obesity, adipose tissue expands to cope with extra nutrients in circulation and avoid lipid deposition in other organs. Unfortunately, this expansion has its limits, and eventually adipose tissue becomes dysfunctional (6).

Another mechanism that has been postulated to contribute to obesity-related metabolic disorders is defective fatty acid oxidation (FAO). Even though some controversy exists about this topic, mainly due to tissue variability and the obesity state of the subjects, there is evidence of a decrease in FAO capacity in humans and rodents during obesity that contributes to lipid accumulation and lipotoxicity (7–14). Strategies that focus on enhancing FAO have been developed to treat obesity with positive results (15–23).

Among obesity-derived adipose tissue dysfunctions, there are two factors that are crucial for the generation of ceramides; key metabolites of sphingolipid (SPL) metabolism that contribute to

obesity-related disorders (24, 25). First, the insulin resistance of obese adipose tissue maintains adipocyte lipolysis on. As a result, FFAs are constantly pumped into circulation. One of the main pathways of ceramide synthesis, the *de novo* pathway, depends on the availability of saturated FFAs (26). Therefore, an increase in saturated FFAs in circulation is a perfect scenario to promote *de novo* ceramide synthesis. Second, adipocyte cell death and dysfunction due to an excess of nutrients generates local inflammation, which promotes immune cell infiltration in the tissue. Then, inflammation is amplified systemically to reach the rest of the body (6, 27). A second ceramide synthesis pathway, the catabolic conversion of another SPL, sphingomyelin (SM), into ceramide by the action of sphingomyelinases, can be activated by inflammatory signals such as TNFα (28). TNFα is a classical cytokine that is elevated in circulation during obesity and known to cause insulin resistance (29, 30). Thus, both elevated saturated FFAs and inflammation, which are key signatures of obesity, promote ceramide synthesis.

Ceramides have been linked to obesity, insulin resistance and metabolic disorders (24, 25). However, most studies have focused on total ceramide levels, rather than the presence of a specific ceramide (31, 32). The lipidomics era has brought the attention to individual ceramide molecular species that are produced via specific pathways and perform distinct functions. Therefore, it is not only a matter of the quantity, but also the quality of ceramide that is modulated in pathological states (33).

Two studies have demonstrated an increase in specific ceramide species (palmitoyl ceramide or C16:0 ceramide) in obese humans and mice that inhibits FAO and negatively regulates insulin signaling and energy expenditure (34, 35). These two independent studies provide a link between obesity, insulin resistance and impaired FAO through ceramide action. In this review, we will

cover the relevance of specific ceramide species, their metabolism, and common obese-related ceramide signaling that leads to insulin resistance. In addition, given recent studies that identify C16:0 ceramide as the species responsible for the metabolic phenotype of obesity through modulation of FAO (34, 35), we will discuss recent findings that link C16:0 ceramide, FAO and obesity.

CERAMIDE METABOLISM

Ceramides are members of the sphingolipid family and are composed of a long-chain sphingoid base, sphingosine, in *N*-linkage to a variety of acyl groups. There are three well-characterized pathways of ceramide production: 1) the *de novo* pathway, which takes place in the endoplasmic reticulum (ER), 2) the sphingomyelinase pathway that converts SM into ceramides in several cellular compartments such as the plasma membrane, lysosomes, Golgi and mitochondria, and 3) the salvage pathway that occurs in lysosomes and endosomes and converts complex sphingolipids into sphingosine, which is reused through reacylation to produce ceramides. In this review, we will focus on *de novo* synthesis (Fig. 1).

Key enzymes of *de novo* ceramide synthesis in an obese state

In the last decade, there have been great advances in knowledge of the key enzymes involved in the *de novo* ceramide biosynthetic pathway. More of the regulatory proteins and enzymes involved in this pathway have been cloned, and the generation of knockout mice showed the physiological functions of these enzymes. Furthermore, new spectroscopic techniques allow researchers to analyze and quantify multiple ceramide species, which yields insights into which species are the most relevant in pathological conditions such as obesity and related diseases.

De novo synthesis starts in the ER by the action of the serine palmitoyltransferase (SPT), the rate-limiting enzyme of sphingolipid synthesis (Fig. 1). This enzyme catalyzes the condensation of serine and palmitoyl-CoA to produce 3-ketosphinganine (36). The product of SPT, 3-ketosphinganine, is reduced by 3-ketosphinganine reductase (37) to generate sphinganine, the substrate for ceramide synthases (CerS). CerS attach acyl-CoAs of different chain lengths to sphinganine to form dihydroceramides, which are converted to ceramides by dihydroceramide desaturase (DES).

We will next analyze the enzymes involved in *de novo* synthesis in an obese state. The main enzymes involved in *de novo* synthesis are:

- SPTs: the hypothesis that high *de novo* ceramide biosynthesis contributes to the pathogenesis of obesity and metabolic syndrome has been tested by several authors (31, 32, 38). They showed that treatment of genetically obese (ob/ob) and high-fat diet-induced obese (DIO) rodent models with myriocin, a specific inhibitor of SPT, decreased circulating ceramides, hepatic steatosis and body weight, and improved insulin resistance. Although blocking ceramide synthesis at the SPTs level seems a promising strategy to ameliorate metabolic syndrome pathogenesis, the complete inhibition of ceramide synthesis may have deleterious effects in the cell, due to the crucial role of ceramides in the formation of other sphingolipid derivatives that are essential to cell membrane function and for diverse intracellular signaling pathways.

- CerS: the discovery of dramatic increases in individual ceramide chain-length species present in the serum of obese mice has increased the interest in this enzyme family (39). Six mammalian CerS (CerS1-CerS6) have been identified. They are codified by six genes, also named *lass* (longevity assurance) due to their homology to the yeast longevity assurance gene *LAG1* (40). *Lass 1-6* gens are located in different chromosomes and their protein products are integral membrane

proteins located in the ER. Interesting recent reviews (41–43) revealed that CerS differ in their: 1) amino acid composition, protein structure and transmembrane topologies, 2) long-chain acyl-CoA specificities and sphingoid base stereospecificity, 3) tissue distribution, 4) transcriptional, post-translational and activity regulation, and 5) biological function (Table 1). These enzymes have emerged as a critical node in phospholipid metabolism. Interestingly, some data suggest that ceramide with a different acyl-chain-length might be associated with cell dysfunction in lipotoxic conditions. C16:0 and C18:0 ceramides are associated with insulin resistance in mice liver (44) and in myotubes from the skeletal muscle of type 2 diabetic patients (45). The identification of putative ceramides at the onset of insulin resistance and in lipotoxicity pushed the research community to carry out many new studies, to discern which CerS is responsible for these events. Recent data obtained from different CerS knockdown showed a high degree of redundancy and inter-regulation between different CerSs (46). Furthermore, knockout mice from CerS1, CerS2, CerS3, CerS4 and CerS5 (47–51) highlight that these enzymes are not only modulators of chain-length in ceramide production, but also control the levels of other bioactive sphingolipids that have different roles depending on the tissue. Data from these studies indicate that CerS5 and CerS6 may be the main CerSs involved in obesity development. New studies are necessary to understand the precise role of each CerS, to discern which ceramide species are toxic in pathological processes such as obesity or insulin resistance, and to develop pharmacological inhibitors of specific CerS to counteract ceramide negative actions.

- **DES:** recently, dihydroceramides have also been considered bioactive lipid species. In obesity there is an imbalance between dihydroceramide/ceramide, and it has been reported that plasma dihydroceramide levels correlate better than ceramides with body mass index (BMI) in cohorts of obese subjects (52, 53). There are two DES1 and 2 enzymes localized in the cytosolic face of ER.

They show different tissue distribution and substrate preferences (37, 54). Studies derived from pharmacological DES1 inhibitors such as Fenretinide indicate that inhibition of this enzyme could be a new strategy to prevent and reduce insulin resistance and obesity (55, 56).

CERAMIDE SIGNALING IN OBESITY

Increasing evidence supports a role for ceramides in the pathogenesis of obesity-induced metabolic disorders. Ceramides have been shown to participate through several mechanisms such as inflammation, apoptosis, ROS, ER stress and autophagy.

Ceramide, together with other stimuli such as fatty acids (FAs), various PKC isoforms, proinflammatory cytokines and oxidative and ER stresses, activate JNK, NF-κB, RAGE and TLR pathways that trigger inflammation and insulin resistance in obesity (57–59). Increases in hepatic and muscle ceramide content have been associated with insulin resistance in obese Zucker rats (66). Ceramide can activate phosphatase 2A that dephosphorylates Akt, and protein kinase C-ζ that prevents Akt phosphorylation. Consequently, the Akt/PKB pathway is blocked, leading to insulin resistance (68–74). In contrast, the insulin-sensitizing hormone adiponectin stimulates ceramidase activity, which enhances ceramide catabolism resulting in increased susceptibility of tissues to insulin, and reduced inflammation and apoptosis (68).

Ceramides have also been shown to alter membrane permeability, inhibit electron transport chain (ETC) intermediates, and promote oxidative stress (25, 69). High levels of ceramides are responsible for pancreatic beta-cell apoptosis mediated by reactive oxygen species (ROS) production and mitochondrial dysfunction (70). Both short and long-chain ceramides were shown to increase ROS production in rat heart and liver mitochondria (71–73). Moreover, studies in beta-cell lines implicate ceramide as both a cause (75) and an effector (76) of ER stress. Inflammation

and ER stress have also been found in the hypothalamus of rats administered on the lateral ventricle of the hypothalamus with exogenous ceramide, which led to obesity caused by impaired energy homeostasis (76). Ceramide is known to be a downstream mediator of ghrelin and leptin signaling in hypothalamus, and increased levels of ceramide promote feeding and body weight gain (77, 78).

Finally, several reports have shown that macroautophagy is induced by ceramides through the participation of CerS1 (79–81). This implicates C18:0 ceramide in targeting mitochondria for autophagic clearance. The depletion of mitochondria by mitophagy leads to a lower FAO capacity, and beyond a certain threshold it can drive the cell to irreversible cellular atrophy (lethal mitophagy) (82).

FATTY ACID OXIDATION AND OBESITY

Obese individuals and those with T2DM are known to have lower FAO rates and lower ETC activity in muscle (7, 12, 13), together with higher glycolytic capacities and increased cellular FA uptake compared to non-obese and non-diabetic individuals (83). This indicates that any strategy that can burn off the excess lipids could potentially be a good approach to treat obesity-induced metabolic disorders.

Several studies have demonstrated the effectiveness of increased FAO to fight against obesity and insulin resistance (15, 16, 18–23, 84–86). While some have focused on indirect enhancement of FAO through acetyl-coA carboxylase (ACC) suppression or malonyl-CoA decarboxylase (MCD) overexpression, a large body of evidence is pointing towards a direct increase in FAO through carnitine palmitoyltransferase (CPT) 1 overexpression as a potential target to improve the obese metabolic phenotype. Malonyl-CoA, which is usually derived from glucose metabolism and is an

intermediate in the FA biosynthetic pathway, regulates FAO by inhibiting CPT1. This makes CPT1 the gatekeeper in mitochondrial FA β-oxidation. Thus, in a situation of energy excess, malonyl-CoA inhibits oxidation and diverts FAs fate into its storage as TG. To date, there are three known CPT1 isoforms, with differential kinetics, malonyl-CoA sensitivity and tissue expression: CPT1A (liver, kidney, intestine, pancreas, ovary and mouse and human WAT), CPT1B (brown adipose tissue, skeletal muscle, heart and rat and human WAT), and CPT1C (brain and testis) (83, 84). The fact that CPT1 regulates FAO makes it a very attractive target to decrease lipid levels and fight against obesity-induced metabolic disorders. It has been shown that obese individuals have decreased visceral WAT CPT1 mRNA and protein levels (85). Interestingly, our group and others have demonstrated that CPT1 overexpression in liver (18, 19, 84), muscle (16, 85, 86), and white adipocytes (20–22) can reduce TG accumulation and improve insulin sensitivity.

ROLE OF CERAMIDES IN MITOCHONDRIAL FAO AND OBESITY

Ceramides are known to promote metabolic disorders, but it was not until recently that two studies identified a specific ceramide, C16:0, as a key negative regulator of insulin sensitivity and FAO in obesity (34, 35). Even though no mechanism of action of C16:0 ceramide was available until now, evidence of its increase during obesity and diabetes can be found in human studies. Increased C16:0 ceramide in human subcutaneous adipose tissue was found and, in female subjects only, it negatively correlated with adiponectin (90). In addition, a subcutaneous and epicardial fat lipid analysis of non-obese, non-diabetic and obese diabetic patients also showed changes in C16:0 ceramide. In subcutaneous fat, C16:0 ceramide was higher in obese diabetic subjects than their non-diabetic counterparts, which indicates that C16:0 ceramide increases significantly with the diabetic but not the obese phenotype. In epicardial fat, C16:0 ceramide is significantly higher in

both non-diabetic and obese diabetic patients than in non-obese subjects. However, among all lipid changes in these tissues, only C16:0 ceramide in subcutaneous fat positively correlated with HOMA-IR (91). These studies strongly suggest a role of C16:0 in obesity and diabetes.

Novel link between C16:0 ceramide and FAO in obesity

Turpin et al. and Raichur et al. identified C16:0 ceramide as a key ceramide that negatively regulates insulin sensitivity, FAO and energy expenditure in obesity (34, 35). C16:0 ceramide is *de novo* synthetized by CerS6 in the ER. Turpin et al. identified increased CerS6 expression in obese human adipose tissue that positively correlated with body mass index (BMI), body fat content, hyperglycemia and insulin resistance. The same pattern was observed in white adipose tissue of HFD-fed mice. Accordingly, acyl-chain ceramide profiles in both obese humans and mice showed increased C16:0 and C18:0 ceramide. Conversely, $CerS6^{-/-}$ mice, which have reduced hepatic and adipose tissue C16:0 ceramide content, are protected from HFD-induced obesity and glucose intolerance, due to increased lipid utilization in brown adipose tissue and liver, which increases whole body energy expenditure (34). At the same time, Raichur et al. published a $CerS2^{+/-}$ mouse model, which is more susceptible to steatohepatitis and insulin resistance. CerS2 is the dominant hepatic CerS isoform and preferentially makes very long-chain ceramides (C22:0, C24:0, C24:1). $CerS2^{+/-}$ upregulates CerS5 and CerS6 expression, increases hepatic C16:0 and C18:0 ceramide, and decreases C24:0 and C24:1 ceramide (35). Moreover, overexpression of CerS6 in primary hepatocytes can reproduce the $CerS2^{+/-}$ phenotype that increases C16:0 ceramide, decreases insulin signaling and promotes oleic acid-induced steatosis (35). Thus, the $CerS2^{+/-}$ model displays a similar phenotype to the obese human and mouse characteristics described by Turpin et al., and the opposite phenotype to the $CerS6^{-/-}$ mouse model. These results indicate that upregulation of CerS6 expression and subsequent increases in specific acyl-chain ceramides are an

important mechanism that contributes to obesity. CerS6 emerges as a new target to treat this problem. However, a recently published article by Gosejacob et al. (48) demonstrates that CerS5 also contributes to C16:0 ceramide synthesis in WAT, skeletal muscle, liver and spleen. In fact, CerS5-deficient mice show reduced weight gain, improved glucose tolerance and reduced WAT inflammation after an HFD challenge. However, this protection was not related to changes in beta oxidation. This approach confirms the role of C16:0 ceramide as a weight-gain promoter lipid and obesity-sensing lipid.

Interestingly, the studies by Turpin et al. and Raichur et al. agree that C16:0 ceramide negatively regulates FAO. However, while Raichur et al. and previous studies by this group demonstrate that C16:0 ceramide impairs β-oxidation through inactivation of complex II and IV of the ETC in the $CerS2^{+/-}$ model (35, 92), Turpin et al. claim that the observed increase in lipid utilization in their $CerS6^{-/-}$ mouse is due to enhanced FAO capacity, regardless of respiratory chain capacity (34). Ceramide action on ETC was previously described (93). However, most of the studies were conducted with short-chain soluble ceramide (71, 73), which is not the most abundant ceramide species in tissues and can exert different actions to the more physiological ceramide species. Nonetheless, some studies focused on the effects of C16:0 ceramide on ETC and demonstrated that C16:0 ceramide inhibits complex IV, which contributes to ROS formation with no effects on mitochondrial membrane potential (72, 92). Oxidative stress is a hallmark of obesity that can inactivate a large number of enzymes. A metabolomics study on HeLa cells revealed that CPT1 is one of the enzymes inhibited by oxidative stress. In this study, they looked at pairs of substrate-product altered by H_2O_2 and other ROS. Among all metabolite changes, the most significant indicated that CPT1 was a major target for oxidative inactivation. Furthermore, CPT1 activity can be recovered by adding catalase to cells. Thus, ROS mediates reversible CPT1 inhibition (94). In

summary, this study provides a unique link between oxidative stress and CPT1 inactivation. These are two scenarios present during obesity that can explain decreased FAO.

With all this information in mind, we can outline a model in which obesity increases saturated FAs and CerS6, leading to C16:0 accumulation. This can cause ETC dysfunction and generate ROS. The ROS can then inactivate CPT1, decreasing FAO, and as a result promote lipid accumulation within the cells (Fig. 2). Some human data can be found in the literature supporting this model. A study of endurance training in obese humans showed a decrease in C16:0 ceramide after training, coupled with an increase in CPT1 activity and FAO in muscle, all of which lead to improved glucose tolerance (95). Exercise training decreases C16:0 ceramide and increases CPT1 activity. Overall, it rescues FAO in human obese skeletal muscle and whole body glucose metabolism.

Unfortunately, it is widely known that lifestyle interventions fail as a treatment for obesity, since they entails patients' long-term commitment. One strategy that could mimic exercise training is enhancing FAO through CPT1 overexpression. Several animal and cellular models have been developed to increase FAO to treat obesity successfully, and some of them showed lower total ceramide content as part of the improved phenotype (16, 18, 85). However, no specific data on ceramide species were available in most of the studies. Only a few studies showed changes in ceramide species after FAO modulation. In an *in vitro* study, enhanced FAO in skeletal muscle cells protected them from palmitate-induced lipotoxicity and insulin resistance, which correlated with a decrease in total ceramide and specifically C16:0 ceramide (17). This study supports the idea that enhancing FAO through CPT1 overexpression might be a good strategy to decrease C16:0 ceramide and avoid its deleterious effects on metabolism in obese states. Although enhancing FAO is a good strategy to rescue C16:0 ceramide actions during obesity, we are aware that C16:0

ceramide has FAO-independent functions in metabolic diseases. Obesity is associated with an increase in endocannabinoid system signaling, which triggers insulin resistance. It has been demonstrated in mice that inhibition of cannabinoid-1 receptor reduces *de novo* ceramide synthesis through a decrease in expression and activity of SPT, CerS1 and CerS6. This leads to a reduction of C16:0 ceramide, among others. These events protect animals from diet-induced body weight gain, hepatic steatosis, glucose and insulin intolerance (44). This study again implicates C16:0 ceramide in metabolic diseases.

CONCLUDING REMARKS

Given the recent findings, research on metabolic diseases should include the role of C16:0 ceramide in these pathologies. Obesity increases C16:0 ceramide (34), and circulating levels of C16:0 ceramide might become a metabolic marker of obesity and associated metabolic dysfunctions. An example can be found in human studies with obese subjects who underwent gastric bypasses. After surgery, obese patients had lower body weight and decreased plasma C16:0 ceramide levels (96, 97). In addition, a decrease in plasma C16:0 ceramide positively correlates with a reduction in plasma TNFα, an inflammatory cytokine that is involved in insulin resistance (97). In animal models, genetic obese ob/ob mice display increased levels of plasma ceramide. Specifically, C16:0 and C18:0 ceramide are higher than in lean mice (98). Altogether, these data suggest that C16:0 ceramide could be used as a metabolic marker of obesity and associated pathologies.

Obesity dysfunctions depend on individual susceptibility. Genetic background differs from individual to individual, predisposing to or protecting from pathologies. C16:0 ceramide could

mediate the transition from the obese to the insulin-resistant phenotype, and gene variants of CerS2, CerS5 or CerS6 could have an impact on C16:0 ceramide levels. We could only find one article on CerS gene variants and metabolic diseases. In this study, a human gene variant of CerS2 was associated with an increase in albuminuria in patients with diabetes, a common condition that indicates progression of the disease (99). No data were provided on the activity of this CerS2 variant or on levels of C16:0 ceramide, but it would be interesting to investigate how many gene variants of CerS2, CerS5 and CerS6 exist in humans, their effects on enzyme activity, and whether they can modulate C16:0 levels and have an impact on metabolic diseases.

As it is known that C16:0 ceramide has a negative impact on metabolism it is crucial to develop specific CerS5 and CerS6 inhibitors to treat obesity and associated comorbidities. This is a difficult task, due to the high homology between ceramide synthases. To the best of our knowledge, only one study attempted to develop specific CerS competitive inhibitors derived from the immunosuppressant Fingolimod (FTY720). Compound ST1072 can inhibit CerS4 and CerS6 (100), but there are no data yet on *in vivo* effects under a HFD challenge. Importantly, the new data on regulation of CerS activity by phosphorylation or deacetylation (101, 102) open up new therapeutic options to control C16:0 ceramide production and its negative effects on health.

The strategy that we, and other labs, have to treat obesity is to enhance FAO. Enhancing FAO through CPT overexpression forces FFAs to enter into mitochondria for oxidation. Ceramide *de novo* synthesis relies on saturated FFA availability. In obesity in particular, palmitic acid is essential for C16:0 ceramide formation. By enhancing FAO, it is possible to 1) reduce overall ceramide formation and 2) kidnap the palmitoyl-coA necessary for C16:0 ceramide generation.

This could reduce the deleterious effects associated with this obesity-related ceramide species. More studies on enhancing FAO with lipidomic data will be needed to prove this concept.

DISCLOSURE

The authors report no conflicts of interest.

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FIGURE LEGENDS

Figure 1. *Sphingolipid/ceramide biosynthetic and remodeling pathways.* There are three main pathways of ceramide generation. 1. The *de novo* pathway takes place in endoplasmic reticulum (ER). Palmitoyl-CoA and serine are condensed by serine palmitoyltransferase (SPT) to form 3-ketodihydrosphingosine. In turn, 3-keto-dihydrosphingosine is reduced to dihydrosphingosine by 3-ketosphinganine reductase (3-KR) to generate sphinganine, the substrate for ceramide synthases (CerS). CerS attach acyl-CoAs with different chain lengths to sphinganine to form dihydroceramides, which are converted to ceramides by DES. 2) The *sphingomyelinase pathway* takes place in the plasma membrane, lysosomes, Golgi and mitochondria and converts sphingomyelin into ceramides bidirectionally. 3) The *salvage pathway* occurs in the late endosomes and the lysosomes and converts long-chain sphingoid bases into ceramides through the action of CerS. SMase: sphingomyelinase; SMS: sphingomyelin synthase; CDase: ceramidase; SPPase: sphingosine phosphate phosphatase; SphK: sphingosine kinase.

Figure 2. *C16:0 ceramide regulates FAO, steatosis and insulin resistance during obesity.* Obesity increase levels of saturated fatty acids (FAs) such as palmitic acid, the limiting substrate of *de novo* ceramide synthesis in the endoplasmic reticulum (ER). Obesity also increases ceramide synthase 6 (CerS6) that is responsible for C16:0 ceramide (C16:0 ceramide) formation, which also depends on palmitic acid availability. C16:0 ceramide can inhibit FA oxidation (FAO) in an electron transport chain (ETC) in an independent or dependent manner, leading to cellular steatosis. ETC dysfunction generates ROS, which can inhibit CPT1 activity and decrease the entry of FA into mitochondria for oxidation. Again, this leads to cellular steatosis. Finally, C16:0 ceramide can inhibit the insulin-signaling pathway, which contributes to obesity-derived insulin resistance.

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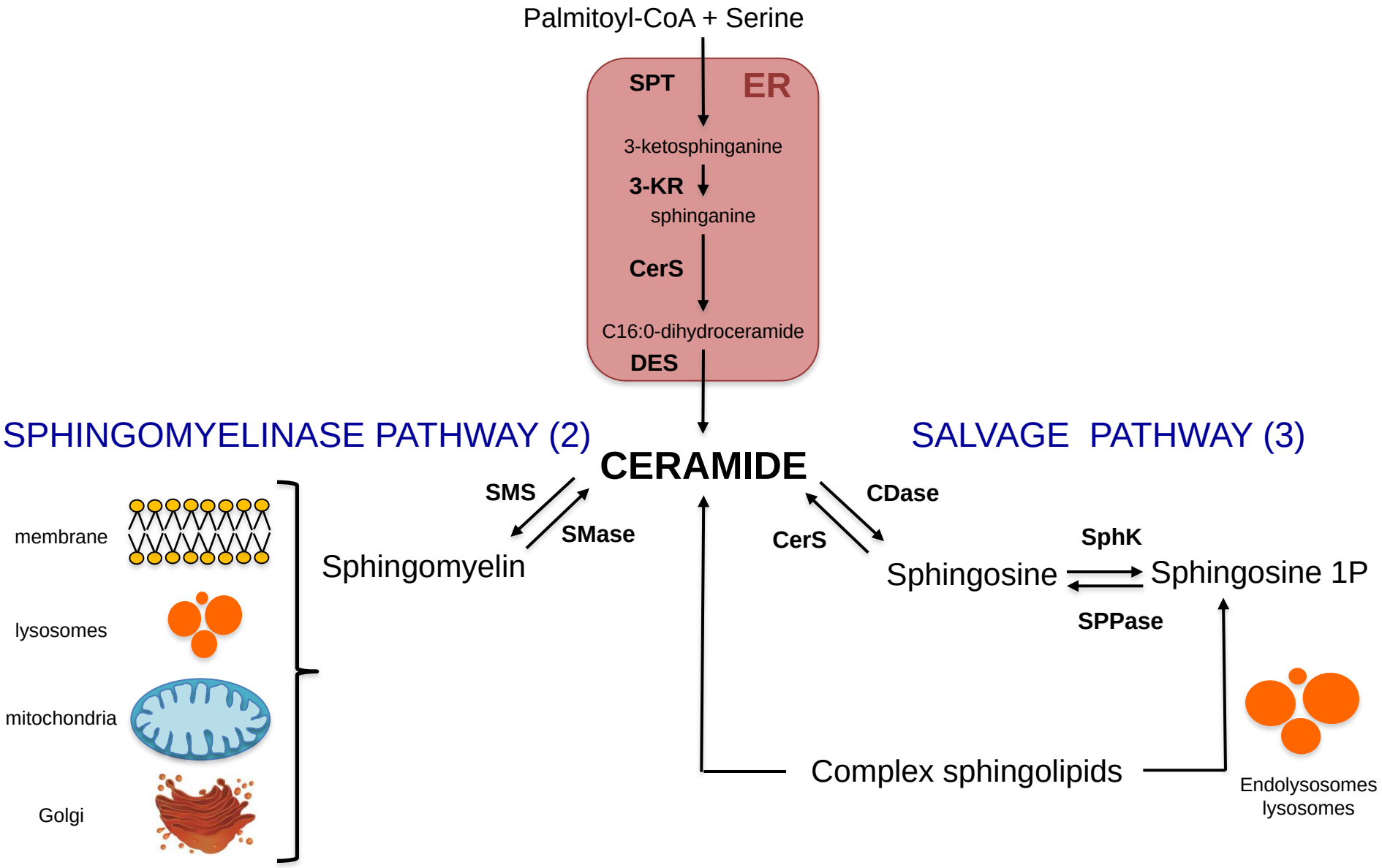
713 **Table 1.** CerS characteristics and functions. Data extracted from (34, 35, 41–43, 103).

Table 1. CerS characteristics and functions. Data extracted from (34, 35, 41–43, 103).

Name	**Protein size** (Da) (human)	**Tissue distribution** (mouse/human)	**Acyl chain-length specificity**	**Implication in cellular processes**	**Mouse models**	**Alterations**
CerS1	39,536	Brain, skeletal muscle, testis	C18	Cerebellar development, Neuronal function	KO mice	Neurodegeneration
CerS2	44,876	Kidney, liver	C22-26	FAO ER stress Autophagy	KO mice	Liver and nervous system dysfunction, obesity, insulin resistance
CerS3	46,217	Testis, skin	C22-26	Spermatogenesis Keratinocyte - differentiation	KO mice	Skin barrier permeability alteration
CerS4	46,399	Skin, heart, liver, leucocytes	C18-20	Hypoxia Apoptosis Stem cell activation	KO mice	Hair loss
CerS5	45,752	WAT, lung, thymus Ubiquitous	C14-16	Hypoxia Apoptosis Autophagy	KO mice Knockdown	Diet-induced obesity
CerS6	44,890	Intestine Ubiquitous	C14-16	FAO ER stress Apoptosis	KO mice Knockdown	Obesity Reduced tumor growth

Figures

FIG. 1



Figures

FIG. 2