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# **Alterations of endogenous sphingolipid metabolism in cardiometabolic diseases: towards novel therapeutic approaches**

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23 ***Highlights***

- 24 • Serum and tissue SL levels are altered in obesity and metabolic diseases.
- 25 • Lipoprotein SL composition and functionality are modified in metabolic diseases.
- 26 • There are novel data about accumulation of SL in cell lipid droplets.
- 27 • Modulation of SL metabolism could be beneficial for metabolic diseases prevention.
- 28 • Dietary SL can partly reach the colon and thereby interact with the gut microbiota.

29 **ABSTRACT**

30 The increasing prevalence of obesity and metabolic diseases is a worldwide public health  
31 concern, and the advent of new analytical technologies has made it possible to highlight the  
32 involvement of some molecules, such as sphingolipids (SL), in their pathophysiology. SL are  
33 constituents of cell membranes, lipoproteins and lipid droplets (LD), and are now considered  
34 as bioactive molecules. Indeed, growing evidence suggests that SL, characterized by diverse  
35 families and species, could represent one of the main regulators of lipid metabolism. There is  
36 an increasing amount of data reporting that plasma SL profile is altered in metabolic diseases.  
37 However, less is known about SL metabolism dysfunction in cells and tissues and how it may  
38 impact the lipoprotein metabolism, its functionality and composition. In cardiometabolic  
39 pathologies, the link between serum SL concentrations and alterations of their metabolism in  
40 various organs and LD is still unclear. Pharmacological approaches have been developed in  
41 order to activate or inhibit specific key enzymes of the SL metabolism, and to positively  
42 modulate SL profile or related metabolic pathways. Nevertheless, little is known about the  
43 long-term impact of such approaches in humans and the current literature still focuses on the  
44 decomposition of the different parts of this complex system rather than performing an  
45 integrated analysis of the whole SL metabolism. In addition, since SL can be provided from  
46 exogenous sources, it is also of interest to evaluate their impact on the homeostasis of  
47 endogenous SL metabolism, which could be beneficial in prevention or treatment of obesity  
48 and related metabolic disorders.

49  
50 **Keywords:** *Obesity, metabolic diseases, sphingolipids, lipoproteins, lipid droplets*

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## 1. Introduction

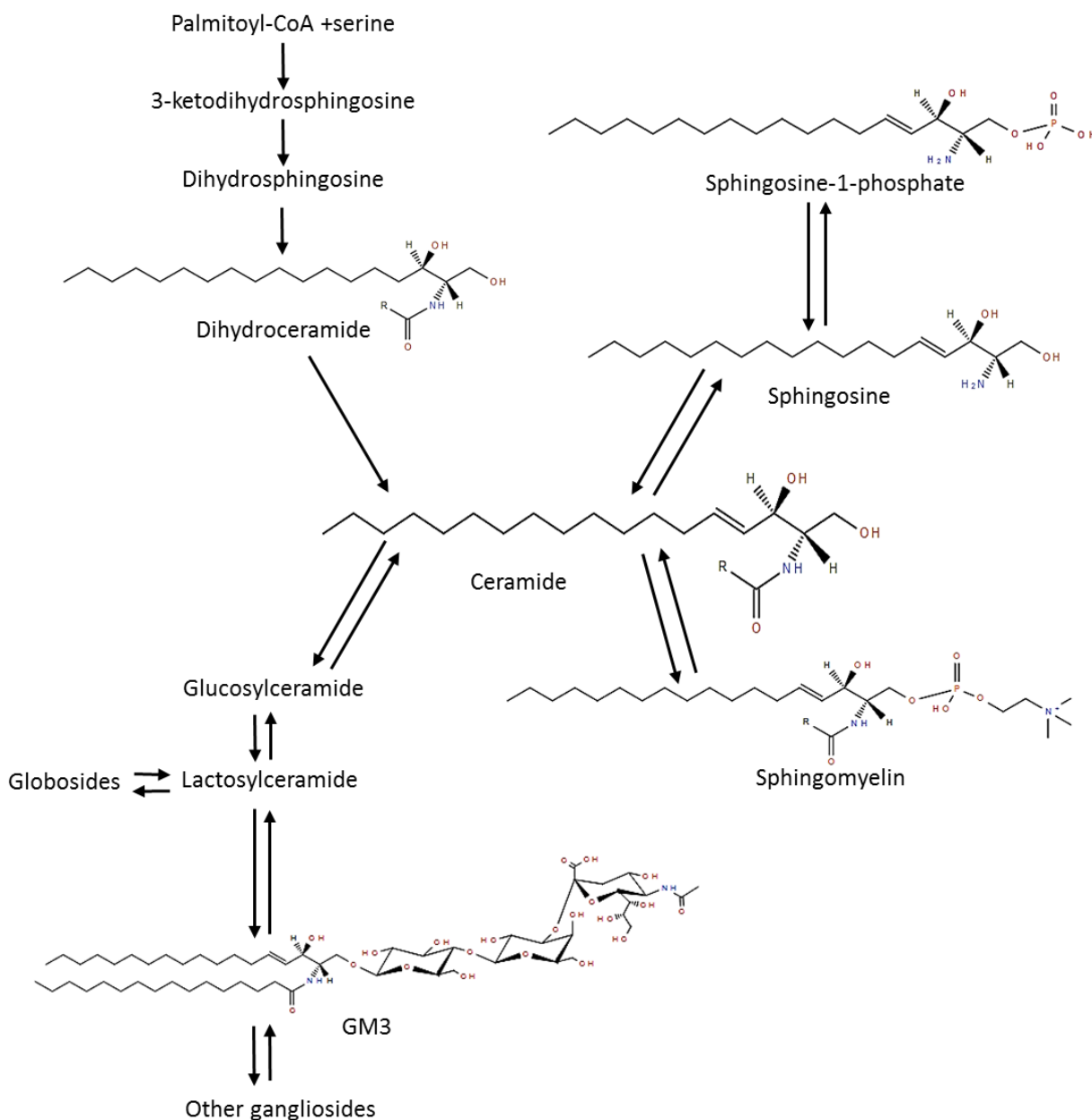
Worldwide incidence of obesity has increased exponentially in the last few years as well as the prevalence of low-grade inflammation associated disorders [1] such as non-alcoholic fatty liver disease (NAFLD) [2], type 2 diabetes (T2D) and cardiovascular diseases (CVD) [3]. Aside from traditional biomarkers of metabolic risks, progress in analytical technologies in the last decades have facilitated the study of other molecules, especially bioactive lipids such as phospholipids and sphingolipids (SL). SL are known as ubiquitous molecules that are important components of cell membranes and lipoproteins [4]. They are involved in many biological processes starting with cell growth, senescence and death as well as cell adhesion and migration, but also inflammation, immune responses and nutrient uptake, among others [5]. It is also starting to emerge that SL could be involved in the pathophysiology of obesity and cardiometabolic risk factors. Indeed, changes in plasma or serum SL concentrations are associated with metabolic disorders as well as accumulation of some SL species in insulin-sensitive tissues, such as the liver [6]. SL could also be involved in the accumulation of an emerging cell organelle that is the lipid droplets (LD), especially in non-adipose tissue [7]. In addition to the endogenous SL metabolism, exogenous SL present in the diet also need to be considered, especially regarding their potential effect on the intestine and the gut microbiota - other important actors of metabolic homeostasis [8]. Another non-negligible aspect is that the liver and the gut are organs that are largely involved in and impacted by metabolic abnormalities. Considering that they are the producers of lipoproteins that carry a great amount of both endogenous and exogenous SL, it is also highly relevant to study the possible effect of an altered SL metabolism on these particles. Indeed, lipoproteins are already known to be highly involved in various metabolic diseases from T2D to atherosclerosis and coronary heart disease but the impact of their SL composition remains largely unknown.

In this review, we will describe the involvement of SL in different pathophysiological mechanisms related to lipid metabolism that could be reflected on systemic SL concentrations and associated with the development of metabolic diseases.

## 2. Overview of sphingolipids metabolism

SL represent a class of bioactive lipids that was first identified in the 19<sup>th</sup> century [9]. Interest for these particular lipids has markedly increased with the study of genetic SL disorders and the identification of complex SL such as cerebrosides or gangliosides. Many elegant reviews

have already detailed SL metabolism to which the interested reader can refer [10–13], therefore this section will provide a summary of the major pathways.



**Figure 1. Summarized partial pathway of sphingolipid biosynthesis**

In this partial metabolic pathway of SL synthesis, general structures of the classes discussed in this review are represented, taking into account that each SL class contains numerous molecular species according to the length of their acyl chain. This Figure contains molecular structures created using the Lipid Structure Drawing Tools from LIPID MAPS online tools for lipid research. Fahy E, Sud M, Cotter D and Subramaniam S. *Nucleic Acids Research* 35, W606-12 (2007).

Briefly, the biosynthesis of SL starts in the endoplasmic reticulum from the condensation of serine and palmitoyl-CoA, and leads to the production of ceramides (Cer) by the introduction of a double bond into its precursor dihydroceramide [14]. Cer represent the basic structure of SL with the presence of a sphingosine base and an acyl chain that can be of various lengths. Each SL class present numerous molecular species according to the number of carbon of this acyl chain (C16, C18, C20, etc.). Sphingomyelin (SM) can be synthesized from Cer by condensation of phosphocholine to a Cer. Of note, SM is also classified as a phospholipid. Finally, more complex molecules comprised in the glycosphingolipid family, such as GM3 ganglioside for example, are constituted of a Cer backbone and various type and number of sugar molecules (**Figure 1**).

In addition to *de novo* synthesis, the generation of Cer from hydrolysis of SM or breakdown of glycosphingolipids can also lead to its conversion into other SL classes or bioactive derivatives), which makes Cer a central molecule of the SL biosynthesis pathway. Cer can be converted back to sphingosine for this base to be recycled into SL synthesis again or phosphorylated to produce sphingosine-1-phosphate (S1P) [15]. SL metabolism is a highly regulated process that can lead to thousands of molecules for which the functions will vary according to their molecular composition and structure (see further details in [16,17,15,18]).

### **3. Plasma alterations of SL metabolism in metabolic diseases**

In the bloodstream, SL are present in the outer cell membranes, in lipoproteins and bound to albumin as well. In healthy individuals, plasma SL are mostly represented by SM (87% of total SL) [4], whereas glycosphingolipids contribute to 9-10% and Cer to 3% of total plasma SL. With few exceptions, detected SL mainly belong to C16, C18 and C24 species in human plasma and tissues [19]. SM is co-localized with cholesterol in cell membranes and all classes of lipoproteins [20] while Cer are equally distributed between ApoB-containing lipoproteins and HDL [19] (Table 1). Glycosphingolipids, mostly glucosylceramides and lactosylceramides, are also present on VLDL, LDL and HDL. Another SL found in plasma and carried in part by lipoproteins is S1P, which is the most abundant SL in HDL [4].

**Table 1. General classification of lipoproteins and distribution of major circulating lipid species.**

	<b>VLDL</b>	<b>LDL</b>	<b>HDL</b>	<b>Total</b>	<b>Ref.</b>
Density (g/ml)	0.93-1.006	1.019-1.063	1.063-1.210		[21]
<b><i>Distribution of major lipid content (% of total content carried by circulating lipoproteins)</i></b>					
TG	66	26	7	100	[22]
Cholesterol esters	20	57	23	100	[22]
Unesterified cholesterol	7	69	24	100	[22]
Phospholipids	7	31	62	100	[23]
<b><i>Distribution of minor lipid content (% of total content carried by circulating lipoproteins)</i></b>					
Phosphatidylcholine	5-8	30-39	56-62	100	[22,23]
Sphingomyelin	3-7	33-50	43-64	100	[19,23]
Ceramides	9-16	40-60	24-51	100	[19,23]
S1P*	1-2	4-6	90-95	100	[19,24]
Glycosphingolipids	6-9	42-50	42-52	100	[24]
<b><i>Protein composition</i></b>					
Apolipoproteins	Apo B-100, Apo E, Apo C	Apo B-100	Apo A-I, A-II, Apo C, Apo E		[21]

\*An important part of total plasma S1P is carried by albumin (30-40%)

### 3. 1. Ceramides and their precursors

In obesity and cardiometabolic diseases, many organs are affected and thus involved in the development of IR and low-grade inflammation, and this can be reflected in systemic circulation [25]. Among SL classes that could be potential biomarkers for metabolic diseases, Cer are the most studied. It is now well established that Cer metabolism is altered in the context of T2D. Many studies have shown an elevation of circulating Cer [26,27] and a positive association with insulin resistance [28] as well as a role of Cer in  $\beta$ -cell apoptosis [29]. Higher plasma concentrations of Cer, as compared to healthy individuals, have even been reported in obese female children and adolescents with T2D and it was correlated with a deleterious metabolic profile [30]. Cer increased plasma levels have also been described in other metabolic diseases or cardiovascular risk factors, such as NAFLD [31], visceral obesity



[32,33], atherosclerosis [34], and are a predictor of cardiovascular mortality [35]. In addition, Peterson *et al.* [36] recently demonstrated that a decreased Cer C24:0/C16:0 ratio could be a more potent predictive marker of coronary heart disease incidence and all-cause mortality, than alterations in specific Cer species.

There is also an increasing interest for other SL classes in the context of metabolic diseases, especially Cer precursors such as dihydroceramides. Indeed, very recent studies suggest that dihydroceramides are not inert molecules as it was first thought, but are also involved in metabolic disturbances [14]. Plasma level of dihydroceramides could even be considered as a potential predictive biomarkers for the development of T2D [37]. For instance, two different clinical studies corroborated results obtained in mouse models by showing a specific increase in plasma long-chain fatty acids (FA)-containing dihydroceramides in individuals who develop metabolic disorders related to obesity and T2D [37,38]. *In vitro* experiments also demonstrated that the accumulation of dihydroceramides inhibits autophagy-related pathways, which in turn could lead to fibrosis and thus plays a role in the progression of NAFLD [39]. It was also revealed that levels of sphingosine and sphinganine are elevated in plasma of patients suffering from T2D, compared to healthy individuals [40]. Regarding S1P, its involvement in cardiometabolic diseases is quite controversial; starting with the fact that a large part (50-70 %) of S1P in circulation is comprised in HDL, which are particles known to have anti-atherogenic effect. However, low S1P concentrations in HDL from T2D or coronary artery disease patients are associated with HDL dysfunctions [41,42]. In the development of atherosclerosis, it is not really defined yet if S1P has beneficial or deleterious effects [43]. Indeed, a recent review from Kurano and Yatomi highlighted that S1P bound to HDL via apolipoprotein M displays atheroprotective properties, while when bound to albumin it exerts both beneficial and harmful effects [44]. Same as for the involvement of S1P in T2D and obesity since it has been reported that S1P plasma levels are increased in these two populations compared to healthy individuals [45,46] are associated with visceral fat accumulation in T2D patients [47]. On the other hand, S1P is thought to have favorable effects in models of lipotoxicity and hyperlipemia as well as for  $\beta$ -cell survival, among others [48].

Interestingly, several studies also reported that serine-palmitoyl-CoA transferase (SPT), a rate-limiting enzyme involved in *de novo* SL synthesis which basically conjugates L-serine and palmitoyl-CoA, is also able to conjugates other amino acid substrates such as L-alanine producing 1-deoxysphingolipids [49–51]. Recent papers demonstrated that this specific SL class could be used as potential predictor of metabolic disorders, such as T2D, since 1-

deoxysphingolipids accumulate in systemic circulation and cells, exerting toxic and pathological effects [51–53].

### 3. 2. Complex sphingolipids

In addition to Cer precursors, complex SL have been of interest when studying cardiometabolic diseases physiopathology. Altered SM plasma levels have also been observed in T2D as well as coronary artery disease and obesity [34,54–56]. Indeed, SM level is considered as an independent risk factor for coronary heart disease [34,57]. Regarding the development of insulin resistance, growing evidence suggests that GM3 is also involved. In fact, the first data about GM3 and insulin response were reported in the early 1990 and a negative correlation was established between GM3 concentrations and insulin responsiveness in leukemic cell lines [58]. Almost two decades later, Sato *et al.* analyzed GM3 concentrations in individuals with hyperlipemia, T2D patients with or without hyperlipemia and controls. They observed that serum GM3 levels were elevated in all groups compared to controls [59]. Lastly, a recent study confirmed a higher concentrations of some GM3 molecular species in subjects with visceral fat accumulation and metabolic disease and showed that hydroxylated GM3 species concentrations in human serum were also increased and correlated with risk factors of cardiometabolic diseases [60].

When examining the relation between SL plasma levels and metabolic diseases, it is hard to draw a clear line in the associations between all the different SL classes and each metabolic disorder. Moreover, it is now necessary to have a better understanding of how SL metabolism is altered in tissues of interest and at the cellular level, notably in LD.

## 4. Modulation of cholesterol and lipid metabolism by sphingolipids in the gut-liver axis

Growing evidence supports that the gut-liver axis plays an essential role in nutrient absorption and NAFLD development [61,62]. Indeed, the development of metabolic disorders has been widely associated with impaired gut homeostasis, gut microbiota dysbiosis and increased intestinal permeability [63]. Recent reviews described how SL modulate metabolic and inflammatory pathways in the gastrointestinal tract [64,65]. However, once ingested, metabolites produced after digestion by enzymes from host and microbial cells present in the

gut, reach the liver through the portal vein and could also exert some physiological or pathological effects.

#### 4. 1. Interplay between intestinal absorption of cholesterol and sphingomyelin

In metabolic diseases, lipid metabolism is altered at several levels from intestinal absorption to endogenous biosynthesis and lipid species distribution, and could participate to the worsening of cardiometabolic disorders. In obese patients with T2D, intestinal absorption of cholesterol is lower than in healthy subjects [66], whereas in normal metabolic conditions, there is already an inter-individual variation of 30 to 80% [67]. Moreover, in metabolic diseases, an altered bile acid profile was associated with hypercholesterolemia and defects in pathways related to lipid metabolism [68]. SL are complex molecules that cannot passively diffuse into enterocytes. Consequently, they need to be hydrolyzed by bile acids and enzymes at the surface of epithelial cells and in the lumen [69]. Once absorbed, SL influence the enterocyte lipid flux: SM is hydrolyzed to sphingosine which is incorporated into triglycerides (TG) after conversion and acetylation, and then reach the lymphatic circulation in chylomicrons [70]. Agren *et al.* demonstrated that postprandial lipid profiles and lipoprotein composition depend on the efficiency of cholesterol absorption, which was determined according to a higher baseline serum cholestenol/cholesterol ratio [71]. In fact, compared to low cholesterol absorbers, high cholesterol absorption efficiency results in a higher concentration of triglycerides, cholesterol and SM in VLDL during the postprandial phase, and a higher free cholesterol/PC ratio in chylomicrons [71]. In rats, dietary supplementation of a mixture of SM and cholesterol demonstrated a mutual inhibitory effect on their absorption, inducing an increased presence of Cer in the colon [72]. In Caco-2 cells, the incorporation of SM in apical bile salt-cholesterol micelles inhibits cholesterol absorption, which could be explained by the decrease of ABC-A1 and -G1 gene expression [73].

#### 4. 2. Ceramide metabolism in liver and its relation with metabolic diseases

In metabolic diseases, excess of lipids (*e.g.* saturated FA) is reflected in the systemic circulation but also in tissues such as liver, which in turn leads to *de novo* Cer synthesis [26]. Liver plays a critical role in the organism homeostasis since it directly participates to detoxification as well as metabolism, biosynthesis and storage. In addition, this important organ has the capacity to repair itself and regenerate. A recent review by Nojima *et al.* focused on the role of SL in both liver injury and regeneration [74]. Moreover, several *in vivo*

and clinical studies reported that the impact of SL on the liver could be linked to their acyl-chain length and composition [75–77]. Indeed, Montgomery *et al.* compared the hepatic profile of Cer and SM in 5 different strains of mice submitted to a high-fat diet. In the 4 mice strains that developed glucose intolerance, the results revealed a marked decrease of very long chain Cer and SM species (C>22) and a significant increase of C16-C22 Cer and SM species in the liver [77]. Interestingly, it was correlated with the specific decrease of Cer synthase 2 (CerS2) expression and activity. Park *et al.* demonstrated that in CerS2 null mice, unable to synthesize Cer C22-C24, the activity of key proteins involved in FA uptake was dysregulated in favor of an increase of CD36 [76]. In clinical studies, a similar pattern was reported with a significant decrease of serum Cer C24 in patients suffering from cirrhosis [75]. In addition, Grammatikos *et al.* also reported an upregulation of serum acid sphingomyelinase in NAFLD patients, which was also associated with an increase of circulating sphingosine and dihydroceramides species, correlated to elevated cholesterol levels [78]. Another possible pathway involves bile acids which plays an important role in the enterohepatic pathway of cholesterol and thus in lipid metabolism. It has been demonstrated that some toxic bile acids (*e.g.* chenodeoxycholic acid, deoxycholic acid, lithocholic acid) are endogenous activators of farnesoid X receptors (FXR) involved in glucose intolerance and insulin resistance through hepatic *de novo* Cer production [79,80]. Besides, elevated plasma concentration of GM3 has been associated with metabolic diseases [59,60], however Choi *et al.* reported that plasma membrane associated GM3 inhibits the secretion of TG-enriched ApoB containing lipoproteins (*i.e.* VLDL and LDL) and could contribute to lower the development of atherosclerosis [81].

As mentioned above, when evaluating the role of the gut-liver axis, it is also important to consider the gut microbiota as an additional metabolically active “organ”, whose composition and functions are altered in metabolic diseases [82–84]. Thus, the composition of the human gut microbiota may also have an impact on circulating SL, since it has been shown that it contains SL-producing bacteria. Same as for mammalian SL, bacterial SL may be detected and used by intestinal endothelial cells and then potentially modulate lipid metabolism [69].

## **5. Involvement of sphingolipids in lipoprotein metabolism and cell lipid droplets**

Another major aspect to consider when addressing the question of SL systemic alterations in metabolic diseases is their important role in lipid and lipoprotein synthesis and regulation. Among many others, Meikle and Summers recently published a state of the art about the involvement of dyslipidemia, particularly related to SL metabolism disruptions, in metabolic disorders associated with obesity [85]. Indeed, SL can modulate genes involved in lipid biosynthesis in mice [86] and their involvement in cholesterol transport has also been demonstrated [87]. In addition, there is a growing interest for the lipoprotein composition in SL that can be modified in metabolic diseases. The development of mass spectrometry-based lipidomics also allowed to identify and quantify many lipid molecular species in lipoproteins [24,88–91]. These particles have an important role in the transport of endogenous and dietary lipids including SL. They are a complex of lipids and proteins all assembled following the same pattern with a central hydrophobic core of TG and cholesterol esters that is surrounded by apolipoproteins phospholipids, SL and unesterified cholesterol forming a membrane and contributing to maintaining the shape of the particles [21,92] (**Table 1**).

## 5. 1. Sphingolipids in lipoprotein composition and function

Modifications in the lipidome of some lipoprotein classes were observed in cardiovascular diseases and T2D [93,94]. This modified lipid composition could affect lipoprotein clearance from plasma and certain enzymes related to lipoprotein metabolism. A study by Redgrave *et al.* where rats were injected with chylomicron-like TG emulsions showed that when the emulsion was stabilized with 100% SM or 50% SM combined with egg yolk phosphatidylcholine, the clearance of CM emulsions from plasma was negligible and very slow, respectively [95]. In comparison, when the emulsion was stabilized with egg yolk phosphatidylcholine alone, clearance was much more important. This study also showed that the liver uptake of triolein and cholesteryl oleate was significantly reduced when the emulsion contained 50% and 100% SM compared to dioleoyl phosphatidylcholine-enriched emulsions [95]. These results were supported by Arimoto *et al.* who also showed delayed clearance of triolein-SM (2:1 molar ratio) lipid emulsion injected in rats. In addition, the incorporation of SM to the emulsion reduced the binding of ApoE, ApoC-II and ApoC-III to the surface lipids of the emulsion [96], which can have functional consequences that would deserve to be investigated.

Modifications in the lipid composition of lipoproteins are of interest since they could potentially affect the functionality of the particles, especially in the context of metabolic diseases. Most studies have focused on LDL and HDL particles, since it is well established

that the first have atherogenic properties and the second are cardioprotective. Ståhlman *et al.* found that major classes of surface lipids including SM and Cer were diminished in HDL from dyslipidemic insulin resistant and T2D women [97]. Interestingly, Denimal *et al.* observed reductions in SM and d18:1 S1P, but not Cer concentrations in HDL2 and HDL3 of patients with metabolic syndrome without insulin resistance [93]. This suggest that HDL composition is already altered in the presence of metabolic syndrome, but insulin resistance and T2D could alter other specific classes of SL in these particles. Boon *et al.* observed an elevation in Cer content of LDL from T2D individuals compared to lean non-diabetic subjects that was reversed with weight loss [98]. The lipid composition of LDL particles can also affect their aggregability, which is an important aspect in the development of atherosclerosis and cardiovascular complications. A recent study showed that there is a strong association between the surface lipid LDL composition and their susceptibility to aggregate. LDL prone to aggregation are enriched in SM and Cer and contained less lysoPC and PC [99]. On a general point of view, changes of SL composition in lipoproteins are mostly observed in the representation of each SL class rather than in the variation of particular molecular species and this particular question has to be explored.

## 5. 2. Sphingolipids in cell lipid droplets

In addition to lipoproteins, it is now important to consider the role of LD in cells which are recognized as dynamic organelles also involved in lipid metabolism as well as many cellular mechanisms [100]. LD are present in almost all type of cells and tissues and their excessive accumulation in various non-adipose tissues has been associated with many metabolic alterations and diseases. Interestingly, Deevska *et al.* have recently described the importance of SL in LD biogenesis and metabolism in a very elegant review [7]. In addition to cell membranes, SL also are found in very low concentrations in LD with cholesterol and TG. In fact, it is now well-admitted that under obesogenic conditions, ectopic fat accumulation in metabolic tissues such as liver or skeletal muscle causes lipotoxicity and is associated with the development of insulin resistance [26,101], meaning that SL in LD could also play a role in lipid metabolism at the tissue level. Recently, Senkal *et al.* elegantly demonstrated the generation of acylceramide from ceramide in LD of HCT116 cell lines and showed that in the liver of mice fed an oleate-enriched high fat diet, acylceramides were generated from Cer and specifically accumulated in LD and this was accompanied by steatosis development [102]. Nevertheless, in the current literature, there is very few data about SL and LD, since SL

profiles and related metabolic pathways are mostly studied at the whole tissue level and not specifically in LD or cell membranes.

## **6. Towards the prevention of metabolic diseases by the modulation of sphingolipid metabolism**

Despite the reported deleterious role of some SL, particularly Cer, it is important to keep in mind that it is not the presence of SL itself that could be harmful for metabolism, but rather the modulation of the quantity and length of the acyl chain of the represented species [103]. In this context, several approaches have been developed in order to positively modify the SL metabolism, with the aim of reducing metabolic disorders.

### **6. 1. Modulation of enzymes of the sphingolipid metabolic pathway**

Many studies reported that modulating the activity of key enzymes involved in SL metabolism – such as ceramidase or CerS activity – via pharmacological or dietary approaches, could have beneficial impacts on SL metabolism and thus improve metabolic disorders induced in a context of obesity or T2D [104,105]. For instance, the specific knock-down of CerS6 in an obese insulin resistant mice model induced an efficient reduction of Cer C16:0 concentration in plasma and hepatic tissue which was associated with an improvement of glucose tolerance and insulin sensitivity [33]. Xia *et al.* developed a transgenic model of mice able to inducibly overexpress acid ceramidase in the liver and demonstrated that activating this pathway prevented hepatic steatosis and insulin sensitivity, associated with a lower hepatic Cer concentration [106]. In ApoE-KO mice, it has been demonstrated that the oral administration of myriocin, a specific inhibitor of SPT, induced a significant decrease of plasma total cholesterol, SM concentrations and atherosclerotic lesions, combined with an increase in plasma HDL cholesterol [107,108]. Another possible pathway involves adiponectin which acts as a regulator of insulin sensitivity and induces ceramidase activity through its receptors AdipoR1/R2, both depending on AMPK, an important kinase involved in energy metabolism and activated by the increase of S1P [109]. The authors demonstrated that the decrease of Cer via the activation of AdipoR1/R2 in hepatocytes or adipocytes (especially Cer C16:0 but also the other Cer species according to the activated receptor isoform) was associated with the improvement of hepatic steatosis, glucose tolerance and insulin sensitivity in mice fed with a high-fat diet [109]. Another potential mechanism concerns the suppression of the intestinal FXR signaling, which in turn induced a decrease of

plasma Cer by reducing the expression of genes of Cer synthesis, thus leading to a decrease of hepatic gluconeogenesis [80].

However, in the literature, both *in vivo* and clinical studies do not reflect the complexity of the SL metabolism since the authors selected and analyzed specific classes of SL or pathways without considering the impact of their intervention on the overall metabolism. In fact, since SL classes and species are differentially distributed among cells, tissues and systemic circulation, it is quite reductive to only describe the modulation of few SL species in one or two compartments of the individual without keeping in mind that the intervention could have an effect in SL profile in other metabolic tissues, specific classes of lipoproteins or in LD at the cellular level. Indeed, further *in vitro* studies are needed to demonstrate potential mechanisms or to suggest an integrative model.

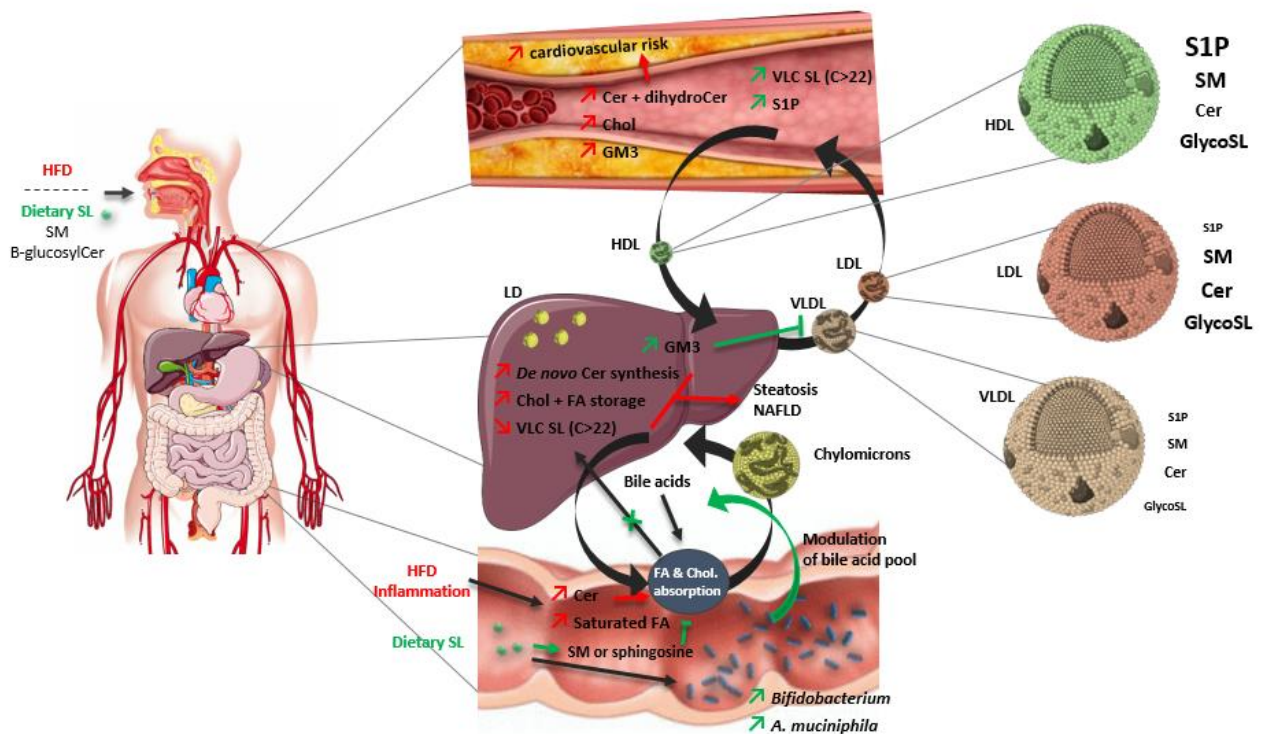
## 6. 2. Beneficial effects of dietary sphingolipid supplementation in metabolic diseases

SL are ubiquitous in many plant and animal organisms, explaining that they are found in non-negligible amounts in our diet. Therefore, the consumption of dietary SL may modulate plasma and tissue levels of SL and thus could impact the endogenous SL metabolism. Indeed, Norris and Blesso recently published a state of the art concerning the potential beneficial effects of dietary SL in (i) the regulation of inflammatory signaling pathways, (ii) the decrease of dyslipidemia focusing on hepatic lipid accumulation and also (iii) the positive modulation of the gut microbiota associated to a decrease of metabolic endotoxemia in mice [110,111].

The major dietary sources of SL studied are chicken eggs, meat and dairy products [112]. The particularity of milk and dairy products is that the milk fat presents a specific profile of bioactive lipids, rich in SM and Cer, and organized in milk fat globule membrane (MFGM) [113–115]. In addition, the consumption of dairy products has been associated with a decrease of metabolic syndrome prevalence or improvements of metabolic disorders related to obesity and CVD [116–118]. Buttermilk is a dairy product particularly rich in milk polar lipids and our group recently revealed its SL composition [119]. In fact, previous *in vivo* studies demonstrated that the consumption of milk polar lipid-rich extract (derived from buttermilk) prevented hepatic TG and cholesterol accumulation normally induced by a high-fat diet [120], and addition of SM in the diet decreased cholesterol absorption in mice [121]. More recently, our group confirmed that milk polar lipids significantly enhance *in vitro* digestive lipolysis and improve postprandial lipid metabolism in mice, compared to soy polar lipids [122]. In a



crossover study, Conway *et al.* demonstrated that buttermilk drink consumption during 8 weeks improved fasting lipid metabolism with a decrease of plasma total- and LDL-cholesterol, as well as TG [123]. A recent clinical study performed in our laboratory by Vors *et al.* [124] corroborated these results by revealing that the 4-week consumption of milk polar lipids concentrated from buttermilk, which are rich in SM, decreased hypercholesterolemia and hyperlipemia both at fasting state and during the postprandial phase, in overweight postmenopausal women at cardiovascular risk. In addition, in overweight individuals, Demmer *et al.* reported that the supplementation of MFGM to a palm oil-rich meal induced a lower plasma cholesterol and LDL concentrations during the 6-hours postprandial phase, compared to the palm oil-rich meal alone [125]. Another promising dietary SL,  $\beta$ -glucosylCer, a natural glycosphingolipid, has been studied in humans. Lalazar *et al.* demonstrated that the daily uptake of  $\beta$ -glucosylceramide during 40 weeks decreased steatosis and improve glucose tolerance in NASH patients, as compared with placebo-receiving individuals [126].



**Figure 2. Schematic representation of potential sphingolipid metabolism alterations in cardiometabolic diseases.** In obesity and cardiometabolic diseases, many tissues are affected and undergo profound metabolic dysfunction. Among them, the metabolism of major lipids like triglycerides can be altered, but the involvement of minor bioactive lipids like SL in these processes is starting to emerge. SL are involved in lipid metabolism regulation in organs such

as the liver and the gut, but is also an important constituent of cell lipid droplets and lipoproteins. Plasma SL concentrations could be a reflection of all the SL metabolism alterations in the gut-liver axis as well as changes in lipoprotein composition in obesity and metabolic diseases. Cer: ceramides, Chol: cholesterol, FA: fatty acids, glycoSL: glycosphingolipids, HFD: high fat diet, LD: lipid droplets, NAFLD: non-alcoholic fatty liver disease, SL: sphingolipids, SM: sphingomyelin, SIP: sphingosine-1-phosphate, VLC: very long chain. This Figure contains pieces of artwork that are derivatives of artworks kindly provided by the Servier Medical Art database licensed under a Creative Commons Attribution 3.0 Unported License.

### 6. 3. Modulations of the gut microbiota composition and cholesterol absorption by sphingolipids

In addition, since the gut microbiota is involved in the development of low-grade inflammation and metabolic disorders [127,128], the use of pharmacological or dietary molecules in order to positively modulate its composition or function has gained more attention. For example, in a rat model of fructose-induced metabolic disorders, Crescenzo *et al.* [129] demonstrated that an antibiotic treatment reversed gut microbiota dysbiosis in fructose-fed rats by positively modulating its composition, and decreases hepatic Cer content and insulin resistance in both liver and visceral adipose tissues. Norris *et al.* reported that milk SL supplementation induced an increase of *Bifidobacterium* in the colon of high fat-fed mice and lowered serum total cholesterol and lipopolysaccharide [130]. In a recent *in vivo* study performed in our laboratory, Milard *et al.* demonstrated that dietary supplementation with milk polar lipids (1.1-1.6% of diet corresponding to 0.25-0.36% of SM) prevented the development of obesity without impacting caloric intake, but was associated with changes in gut microbiota populations [131]. Under a more mechanistical approach realized both *in vitro* and *in vivo*, our results demonstrated that SM could induce beneficial effects at the intestinal barrier level through the increase of plasma IL-8, an interleukin involved in immunity, leading to an improvement of gut integrity [132]. These results from Milard *et al.* also suggested that some effects could be mediated by sphingosine, which is liberated during the digestion of SM from both egg and milk [132]. In fact, since SM co-localize with cholesterol, it could explain that dietary SM is associated with a decrease in cholesterol absorption in animal models [121]. However, *in vitro* experiments realized by Garmy *et al.* also demonstrated that

sphingosine forms lipid complexes with cholesterol and this specific interaction inhibits the intestinal transport of micellar cholesterol [133].

#### 6. 4. Sphingolipid metabolism in specific bacterial species

In the previous section, we demonstrated that SL and the gut microbiota could be interconnected since specific dietary SL may modulate the gut microbiota composition. However, it has also been reported that specific bacterial species are able to produce or metabolize SL. In fact, it was previously established that SL were exclusively found in eukaryote cells until their discovery in prokaryotes [134]. For example, whereas most bacterial membranes only contain glycerol-based PL, few of them possess both PL and SL [135]. Among the SL-containing bacteria, the genus *Bacteroides* has been mainly studied because of its specific PL membrane, which is composed of 40-70% of SL [136]. An *et al.* demonstrated that SL-rich membrane of *Bacteroides* plays an important role in the survival of this genus in the gastro-intestinal tract [135]. Wieland Brown *et al.* also reported that  $\alpha$ -galactosylceramides produced by *Bacteroides* species can modulate natural killer T cell activity and thus influence the host immune response [136]. In addition, the genus *Bacteroides* is mainly deprived from key SL metabolism-related enzymes as sphingomyelinases whereas they are found in some pathogens such as *Staphylococcus aureus* [137]. More recently, promising studies demonstrated that, among all the known mechanisms, the anti-inflammatory effects of certain probiotics could be the result of the SL metabolism modulations. Angulo *et al.* reported that *Lactobacillus brevis* and *Streptococcus thermophilus* species could influence the mucosal immune cells by inducing cell apoptosis through the activation of a neutral SMase and the production of Cer [138]. In addition, Soo *et al.* demonstrated that the supplementation with the probiotic cocktail VSL#3 induced a significant increase of alkaline SMase levels in the intestinal sections of both IL-10 deficient mice and humans with colitis, resulting in a decrease of intestinal inflammation [139].

## 7. Conclusion

In this review, we realized a focused state of the art about the possible alterations of the SL metabolism in the context of obesity and associated metabolic diseases. In fact, all the data discussed suggest that SL metabolism is highly involved in the regulation of lipid homeostasis. In addition, changes in both the quantity and profile of represented SL species and classes at the molecular, cellular and tissue levels could also be reflected in circulation

(**Figure 2**). As described above, the most studied pathway mainly concerns the metabolism of Cer whereas few papers now also included the analysis of SM, dihydroceramide, ganglioside and other glucosylceramide species. In clinical studies, the difficult access to biopsies may also be a limitation in the study of the SL metabolism in obese and T2D patients for example, partly explaining why lipidomic analysis are most of the time only realized in systemic circulation. In this review, we discussed about how plasma SL alterations can reflect the accumulation of perturbations in tissue, lipoprotein and LD metabolism. In addition, the last part of this review demonstrated that more and more studies now describe the beneficial impact of metabolic disorder treatments on SL profile and metabolism. These preventive or curative treatments concern pharmacological drugs but also dietary bioactive molecules or extracts. In addition, such as highlighted by Bellini *et al.* [140], it is unthinkable to non-specifically inhibit key enzymes involved in the production of specific Cer species to improve T2D-related metabolic disorders because all SL are part of a plethora of physiological pathways. There is also growing evidence *in vivo* that the dietary supplementation with specific SL could exert beneficial effects on both metabolic disorders and SL metabolism. Therefore, it confers an advantage to prevent or attenuate metabolic disorders development, and to improve overall SL metabolism homeostasis.

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