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2 **Alterations of endogenous sphingolipid metabolism**
3 **in cardiometabolic diseases: towards novel**
4 **therapeutic approaches**

5
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22

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*

51

52 **1. Introduction**

53 Worldwide incidence of obesity has increased exponentially in the last few years as well as
54 the prevalence of low-grade inflammation associated disorders [1] such as non-alcoholic fatty
55 liver disease (NAFLD) [2], type 2 diabetes (T2D) and cardiovascular diseases (CVD) [3].
56 Aside from traditional biomarkers of metabolic risks, progress in analytical technologies in
57 the last decades have facilitated the study of other molecules, especially bioactive lipids such
58 as phospholipids and sphingolipids (SL). SL are known as ubiquitous molecules that are
59 important components of cell membranes and lipoproteins [4]. They are involved in many
60 biological processes starting with cell growth, senescence and death as well as cell adhesion
61 and migration, but also inflammation, immune responses and nutrient uptake, among others
62 [5]. It is also starting to emerge that SL could be involved in the pathophysiology of obesity
63 and cardiometabolic risk factors. Indeed, changes in plasma or serum SL concentrations are
64 associated with metabolic disorders as well as accumulation of some SL species in insulin-
65 sensitive tissues, such as the liver [6]. SL could also be involved in the accumulation of an
66 emerging cell organelle that is the lipid droplets (LD), especially in non-adipose tissue [7]. In
67 addition to the endogenous SL metabolism, exogenous SL present in the diet also need to be
68 considered, especially regarding their potential effect on the intestine and the gut microbiota -
69 other important actors of metabolic homeostasis [8]. Another non-negligible aspect is that the
70 liver and the gut are organs that are largely involved in and impacted by metabolic
71 abnormalities. Considering that they are the producers of lipoproteins that carry a great
72 amount of both endogenous and exogenous SL, it is also highly relevant to study the possible
73 effect of an altered SL metabolism on these particles. Indeed, lipoproteins are already known
74 to be highly involved in various metabolic diseases from T2D to atherosclerosis and coronary
75 heart disease but the impact of their SL composition remains largely unknown.

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

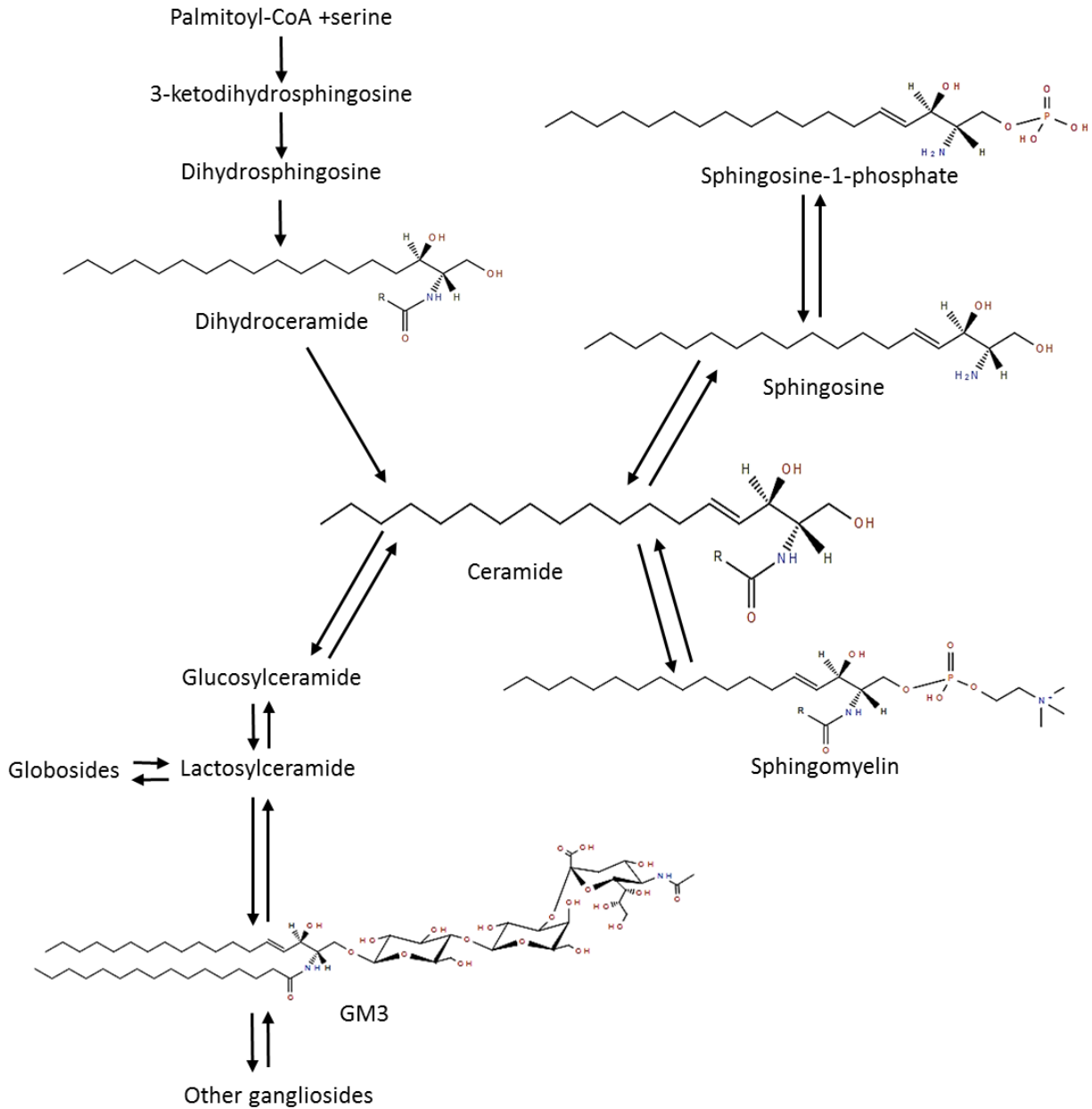
79

80 **2. Overview of sphingolipids metabolism**

81 SL represent a class of bioactive lipids that was first identified in the 19th century [9]. Interest
82 for these particular lipids has markedly increased with the study of genetic SL disorders and
83 the identification of complex SL such as cerebroside or ganglioside. Many elegant reviews

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

86



87

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

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

95

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

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

113

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

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

124

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

	VLDL	LDL	HDL	Total	<i>Ref.</i>
Density (g/ml)	0.93-1.006	1.019-1.063	1.063-1.210		[21]
<i>Distribution of major lipid content (% of total content carried by circulating lipoproteins)</i>					
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]
<i>Distribution of minor lipid content (% of total content carried by circulating lipoproteins)</i>					
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]
<i>Protein composition</i>					
Apolipoproteins	Apo B-100, Apo E, Apo C	Apo B-100	Apo A-I, A-II, Apo C, Apo E		[21]

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

128

129 3. 1. Ceramides and their precursors

130 In obesity and cardiometabolic diseases, many organs are affected and thus involved in the
 131 development of IR and low-grade inflammation, and this can be reflected in systemic
 132 circulation [25]. Among SL classes that could be potential biomarkers for metabolic diseases,
 133 Cer are the most studied. It is now well established that Cer metabolism is altered in the
 134 context of T2D. Many studies have shown an elevation of circulating Cer [26,27] and a
 135 positive association with insulin resistance [28] as well as a role of Cer in β -cell apoptosis
 136 [29]. Higher plasma concentrations of Cer, as compared to healthy individuals, have even
 137 been reported in obese female children and adolescents with T2D and it was correlated with a
 138 deleterious metabolic profile [30]. Cer increased plasma levels have also been described in
 139 other metabolic diseases or cardiovascular risk factors, such as NAFLD [31], visceral obesity

140 [32,33], atherosclerosis [34], and are a predictor of cardiovascular mortality [35]. In addition,
141 Peterson *et al.* [36] recently demonstrated that a decreased Cer C24:0/C16:0 ratio could be a
142 more potent predictive marker of coronary heart disease incidence and all-cause mortality,
143 than alterations in specific Cer species.

144 There is also an increasing interest for other SL classes in the context of metabolic diseases,
145 especially Cer precursors such as dihydroceramides. Indeed, very recent studies suggest that
146 dihydroceramides are not inert molecules as it was first thought, but are also involved in
147 metabolic disturbances [14]. Plasma level of dihydroceramides could even be considered as a
148 potential predictive biomarkers for the development of T2D [37]. For instance, two different
149 clinical studies corroborated results obtained in mouse models by showing a specific increase
150 in plasma long-chain fatty acids (FA)-containing dihydroceramides in individuals who
151 develop metabolic disorders related to obesity and T2D [37,38]. *In vitro* experiments also
152 demonstrated that the accumulation of dihydroceramides inhibits autophagy-related pathways,
153 which in turn could lead to fibrosis and thus plays a role in the progression of NAFLD [39]. It
154 was also revealed that levels of sphingosine and sphinganine are elevated in plasma of
155 patients suffering from T2D, compared to healthy individuals [40]. Regarding S1P, its
156 involvement in cardiometabolic diseases is quite controversial; starting with the fact that a
157 large part (50-70 %) of S1P in circulation is comprised in HDL, which are particles known to
158 have anti-atherogenic effect. However, low S1P concentrations in HDL from T2D or coronary
159 artery disease patients are associated with HDL dysfunctions [41,42]. In the development of
160 atherosclerosis, it is not really defined yet if S1P has beneficial or deleterious effects [43].
161 Indeed, a recent review from Kurano and Yatomi highlighted that S1P bound to HDL via
162 apolipoprotein M displays atheroprotective properties, while when bound to albumin it exerts
163 both beneficial and harmful effects [44]. Same as for the involvement of S1P in T2D and
164 obesity since it has been reported that S1P plasma levels are increased in these two
165 populations compared to healthy individuals [45,46] are associated with visceral fat
166 accumulation in T2D patients [47]. On the other hand, S1P is thought to have favorable
167 effects in models of lipotoxicity and hyperlipemia as well as for β -cell survival, among others
168 [48].

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

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

176

177 3. 2. Complex sphingolipids

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

192

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

197

198 **4. Modulation of cholesterol and lipid metabolism by sphingolipids in the** 199 **gut-liver axis**

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

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

208

209 4. 1. Interplay between intestinal absorption of cholesterol and sphingomyelin

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

231

232 4. 2. Ceramide metabolism in liver and its relation with metabolic diseases

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

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

261

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

268

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

271 Another major aspect to consider when addressing the question of SL systemic alterations in
272 metabolic diseases is their important role in lipid and lipoprotein synthesis and regulation.
273 Among many others, Meikle and Summers recently published a state of the art about the
274 involvement of dyslipidemia, particularly related to SL metabolism disruptions, in metabolic
275 disorders associated with obesity [85]. Indeed, SL can modulate genes involved in lipid
276 biosynthesis in mice [86] and their involvement in cholesterol transport has also been
277 demonstrated [87]. In addition, there is a growing interest for the lipoprotein composition in
278 SL that can be modified in metabolic diseases. The development of mass spectrometry-based
279 lipidomics also allowed to identify and quantify many lipid molecular species in lipoproteins
280 [24,88–91]. These particles have an important role in the transport of endogenous and dietary
281 lipids including SL. They are a complex of lipids and proteins all assembled following the
282 same pattern with a central hydrophobic core of TG and cholesterol esters that is surrounded
283 by apolipoproteins phospholipids, SL and unesterified cholesterol forming a membrane and
284 contributing to maintaining the shape of the particles [21,92] (**Table 1**).

285

286 5. 1. Sphingolipids in lipoprotein composition and function

287 Modifications in the lipidome of some lipoprotein classes were observed in cardiovascular
288 diseases and T2D [93,94]. This modified lipid composition could affect lipoprotein clearance
289 from plasma and certain enzymes related to lipoprotein metabolism. A study by Redgrave *et*
290 *al.* where rats were injected with chylomicron-like TG emulsions showed that when the
291 emulsion was stabilized with 100% SM or 50% SM combined with egg yolk
292 phosphatidylcholine, the clearance of CM emulsions from plasma was negligible and very
293 slow, respectively [95]. In comparison, when the emulsion was stabilized with egg yolk
294 phosphatidylcholine alone, clearance was much more important. This study also showed that
295 the liver uptake of triolein and cholesteryl oleate was significantly reduced when the emulsion
296 contained 50% and 100% SM compared to dioleoyl phosphatidylcholine-enriched emulsions
297 [95]. These results were supported by Arimoto *et al.* who also showed delayed clearance of
298 triolein-SM (2:1 molar ratio) lipid emulsion injected in rats. In addition, the incorporation of
299 SM to the emulsion reduced the binding of ApoE, ApoC-II and ApoC-III to the surface lipids
300 of the emulsion [96], which can have functional consequences that would deserve to be
301 investigated.

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

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

321

322 5. 2. Sphingolipids in cell lipid droplets

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

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

340

341 **6. Towards the prevention of metabolic diseases by the modulation of** 342 **sphingolipid metabolism**

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

348

349 **6. 1. Modulation of enzymes of the sphingolipid metabolic pathway**

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

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

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

382

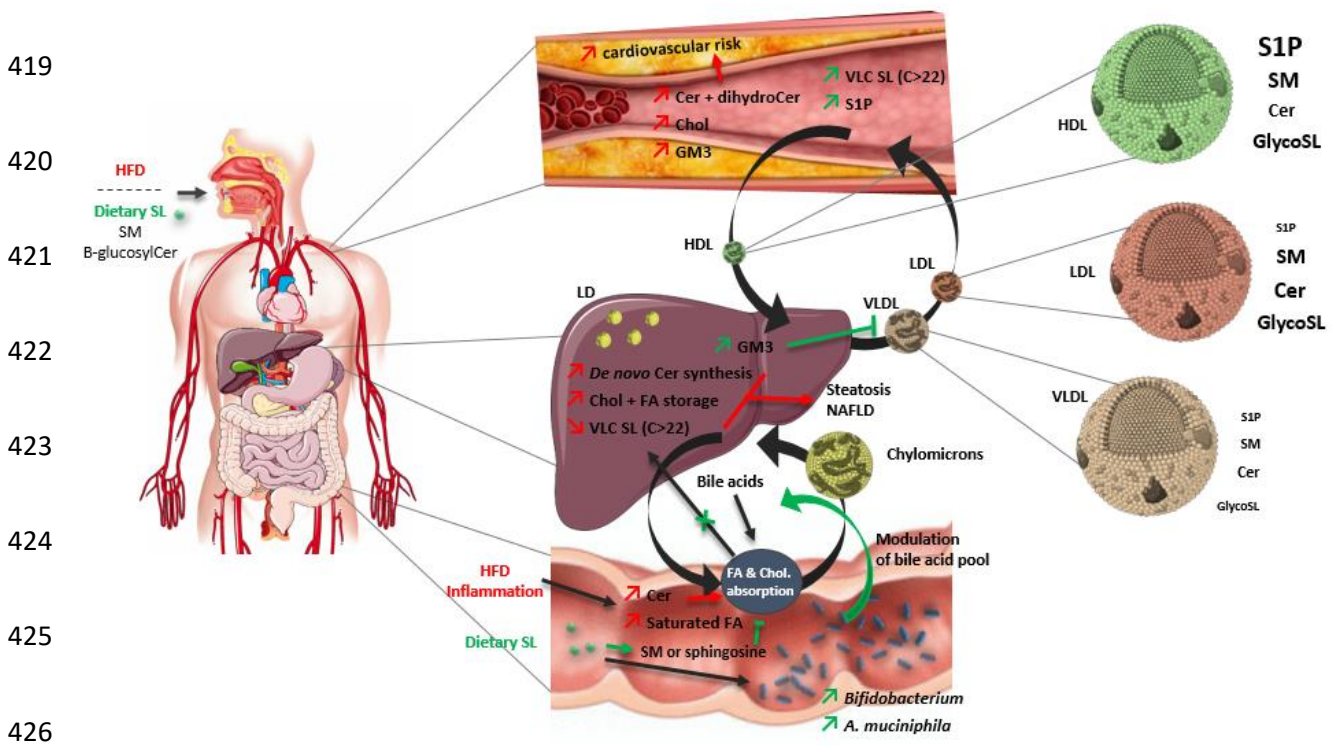
383 6. 2. Beneficial effects of dietary sphingolipid supplementation in metabolic 384 diseases

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

392 The major dietary sources of SL studied are chicken eggs, meat and dairy products [112]. The
393 particularity of milk and dairy products is that the milk fat presents a specific profile of
394 bioactive lipids, rich in SM and Cer, and organized in milk fat globule membrane (MFGM)
395 [113–115]. In addition, the consumption of dairy products has been associated with a decrease
396 of metabolic syndrome prevalence or improvements of metabolic disorders related to obesity
397 and CVD [116–118]. Buttermilk is a dairy product particularly rich in milk polar lipids and
398 our group recently revealed its SL composition [119]. In fact, previous *in vivo* studies
399 demonstrated that the consumption of milk polar lipid-rich extract (derived from buttermilk)
400 prevented hepatic TG and cholesterol accumulation normally induced by a high-fat diet [120],
401 and addition of SM in the diet decreased cholesterol absorption in mice [121]. More recently,
402 our group confirmed that milk polar lipids significantly enhance *in vitro* digestive lipolysis
403 and improve postprandial lipid metabolism in mice, compared to soy polar lipids [122]. In a

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

418



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

432 *as the liver and the gut, but is also an important constituent of cell lipid droplets and*
433 *lipoproteins. Plasma SL concentrations could be a reflection of all the SL metabolism*
434 *alterations in the gut-liver axis as well as changes in lipoprotein composition in obesity and*
435 *metabolic diseases. Cer: ceramides, Chol: cholesterol, FA: fatty acids, glycoSL:*
436 *glycosphingolipids, HFD: high fat diet, LD: lipid droplets, NAFLD: non-alcoholic fatty liver*
437 *disease, SL: sphingolipids, SM: sphingomyelin, SIP: sphingosine-1-phosphate, VLC: very*
438 *long chain. This Figure contains pieces of artwork that are derivatives of artworks kindly*
439 *provided by the Servier Medical Art database licensed under a Creative Commons Attribution*
440 *3.0 Unported License.*

441

442 6. 3. Modulations of the gut microbiota composition and cholesterol absorption 443 by sphingolipids

444 In addition, since the gut microbiota is involved in the development of low-grade
445 inflammation and metabolic disorders [127,128], the use of pharmacological or dietary
446 molecules in order to positively modulate its composition or function has gained more
447 attention. For example, in a rat model of fructose-induced metabolic disorders, Crescenzo *et*
448 *al.* [129] demonstrated that an antibiotic treatment reversed gut microbiota dysbiosis in
449 fructose-fed rats by positively modulating its composition, and decreases hepatic Cer content
450 and insulin resistance in both liver and visceral adipose tissues. Norris *et al.* reported that milk
451 SL supplementation induced an increase of *Bifidobacterium* in the colon of high fat-fed mice
452 and lowered serum total cholesterol and lipopolysaccharide [130]. In a recent *in vivo* study
453 performed in our laboratory, Milard *et al.* demonstrated that dietary supplementation with
454 milk polar lipids (1.1-1.6% of diet corresponding to 0.25-0.36% of SM) prevented the
455 development of obesity without impacting caloric intake, but was associated with changes in
456 gut microbiota populations [131]. Under a more mechanistical approach realized both *in vitro*
457 and *in vivo*, our results demonstrated that SM could induce beneficial effects at the intestinal
458 barrier level through the increase of plasma IL-8, an interleukin involved in immunity, leading
459 to an improvement of gut integrity [132]. These results from Milard *et al.* also suggested that
460 some effects could be mediated by sphingosine, which is liberated during the digestion of SM
461 from both egg and milk [132]. In fact, since SM co-localize with cholesterol, it could explain
462 that dietary SM is associated with a decrease in cholesterol absorption in animal models
463 [121]. However, *in vitro* experiments realized by Garmy *et al.* also demonstrated that

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

466

467 6. 4. Sphingolipid metabolism in specific bacterial species

468 In the previous section, we demonstrated that SL and the gut microbiota could be
469 interconnected since specific dietary SL may modulate the gut microbiota composition.
470 However, it has also been reported that specific bacterial species are able to produce or
471 metabolize SL. In fact, it was previously established that SL were exclusively found in
472 eukaryote cells until their discovery in prokaryotes [134]. For example, whereas most
473 bacterial membranes only contain glycerol-based PL, few of them possess both PL and SL
474 [135]. Among the SL-containing bacteria, the genus *Bacteroides* has been mainly studied
475 because of its specific PL membrane, which is composed of 40-70% of SL [136]. An *et al.*
476 demonstrated that SL-rich membrane of *Bacteroides* plays an important role in the survival of
477 this genus in the gastro-intestinal tract [135]. Wieland Brown *et al.* also reported that α -
478 galactosylceramides produced by *Bacteroides* species can modulate natural killer T cell
479 activity and thus influence the host immune response [136]. In addition, the genus
480 *Bacteroides* is mainly deprived from key SL metabolism-related enzymes as
481 sphingomyelinases whereas they are found in some pathogens such as *Staphylococcus aureus*
482 [137]. More recently, promising studies demonstrated that, among all the known mechanisms,
483 the anti-inflammatory effects of certain probiotics could be the result of the SL metabolism
484 modulations. Angulo *et al.* reported that *Lactobacillus brevis* and *Streptococcus thermophilus*
485 species could influence the mucosal immune cells by inducing cell apoptosis through the
486 activation of a neutral SMase and the production of Cer [138]. In addition, Soo *et al.*
487 demonstrated that the supplementation with the probiotic cocktail VSL#3 induced a
488 significant increase of alkaline SMase levels in the intestinal sections of both IL-10 deficient
489 mice and humans with colitis, resulting in a decrease of intestinal inflammation [139].

490

491 7. Conclusion

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

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

514

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532

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