

# Intestinal Availability and Metabolic Effects of Dietary Camelina Sphingolipids during the Metabolic Syndrome Onset in Mice

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1	Imavailability and metabolic effects of dietary camelina sphingolipids during the metabolic
2	syndrome onset in mice.
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20 **ABSTRACT.** 

Sphingolipids appear as a promising class of components susceptible to prevent the onset of 21 the metabolic syndrome (MetS). Gut availability and effects of *Camelina sativa* sphingolipids 22 23 were investigated in a mouse model of dietary-induced metabolic syndrome. Seed meals from two Camelina sativa lines enriched respectively in C24- and C16- NH<sub>2</sub>-glycosyl-inositol-24 25 phosphoryl-ceramides (NH<sub>2</sub>GIPC) were used in hypercaloric diets. After 5 weeks on these two hypercaloric diets, two markers of the MetS were alleviated (adiposity and insulin 26 resistance) as well as inflammation markers and colon barrier dysfunction. A more 27 pronounced effect was observed with the C16-NH<sub>2</sub>GIPC-enriched HC diet, in particular for 28 colon barrier function. Despite a lower digestibility, C16-NH<sub>2</sub>GIPC were more prevalent in 29 30 the intestine wall. Sphingolipids provided as camelina meal can therefore counteract some deleterious effects of a hypercaloric diet in mice at the intestinal and systemic levels. 31 Interestingly, these beneficial effects seem partly dependent on sphingolipid acyl chain 32 length. 33

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37 **Key words**: sphingolipid, *Camelina sativa*, metabolic syndrome, digestibility, acyl chain.

#### 38 INTRODUCTION

The metabolic syndrome (MetS) is a cluster of abnormalities including visceral obesity, dyslipidemia, hyperglycemia, and increased blood pressure, which results in a higher risk of cardiovascular diseases and type-2 diabetes <sup>(1)</sup>.

Its prevalence is increasing worldwide and makes it a major public health challenge. This 42 increase is associated with several features of lifestyle such as physical inactivity, 43 psychosocial stress, environmental contaminants, and, as a major determinant, dietary 44 habits <sup>(2)</sup>. High caloric intake, typical of Western-type diet rich in saturated fatty acids (SFA) 45 and sugars, is a major causative factor of the onset of the MetS, which is characterized by 46 early events such insulin resistance (IR) and a low-grade inflammation (LGI) (3, 4). These 47 alterations are known to originate, at least partly, from an allostatic stress due to the 48 Western-type diet, which is characterized by microbial dysbiosis <sup>(5)</sup>, together with local 49 inflammation <sup>(6)</sup> and physiological dysfunction of the intestinal wall <sup>(3)</sup>. The resulting 50 endotoxemia enhances the low-grade inflammation (LGI) at the systemic level, which, in 51 turn, amplifies the initiation of the MetS<sup>(7, 8)</sup> In addition to the rodent studies, both 52 observation and intervention human studies confirmed the link between nutrients, gut 53 dysfunction, LGI and initiation of the MetS, and in particular IR<sup>(8, 9)</sup>. 54

In consequence, new preventive and therapeutic strategies for MetS, including lifestylebased changes involving nutrition, must be established, tested and improved in specifically susceptible and vulnerable people, such as the at-risk, obese subjects. In these patients, the challenge is to prevent or delay the onset of IR and low-grade inflammation, and of cardiometabolic complications. Among the different possible solutions are diet changes and/or diet complementation.

As part of dietary candidates susceptible to prevent or alleviate the onset of the MetS, and

62 particularly of IR, sphingolipids (SL) appear as a new and promising class of components. Indeed, body SL originate from endogenous synthesis, but may also be provided by the diet 63 from animal or vegetal products. SL are composed of a sphingoid base (or long-chain base, 64 LCB) acylated by a long or very long-chain FA and bearing a more or less complex polar head 65 group <sup>(10)</sup>. They are essential components of cell membranes and are involved in cell 66 signalling in both plants and animals. They are associated, together with animal cholesterol 67 or plant phytosterols, into membrane micro-domains (caveolae and lipid rafts) <sup>(11)</sup>. These 68 micro-domains are more ordered and less fluid than the rest of the plasma membrane, 69 which favours docking, clustering and functionality of insulin receptor and other membrane 70 receptors <sup>(11, 12)</sup>. Interestingly, the length of the SL acyl chain is an important structural 71 feature modulating the properties of these micro-domains and subsequent translocation 72 and downstream signalling of insulin receptor <sup>(13)</sup>. A number of disparate, but convincing 73 data, suggest that some plant SL (phytoSL) would improve some features of the MetS, such 74 as dyslipidemia <sup>(14, 15)</sup>, IR/glucose intolerance <sup>(15-18)</sup> and non-alcoholic steatohepatitis <sup>(14, 18-20)</sup>. 75 There is also some evidence that dietary SL from both animal and plant origin may exert 76 anti-inflammatory effects at the extra-digestive level, including in the postprandial state (21, 77 22) 78

Other beneficial effects of phytoSL have been described at the intestine level, in mice models of chemically-induced colorectal cancer or inflammatory bowel diseases <sup>(23-28)</sup>. In contrast, when it comes to nutritionally-induced rather than chemically-induced, allostatic intestinal stress, it remains unknown if dietary phytoSL may alleviate microbial dysbiosis and/of gut barrier function alterations, which could explain some beneficial systemic consequences.

85 The mechanisms by which phytoSL exert their beneficial effects are still not elucidated and

since it is a large and diversified lipid family, the role of the different structural components (polar head, acyl chains, long chain base) remains to be deciphered. Indeed, plants have a large variety of SL with more than 300 molecular species, some of which being tissue specific (29). They are characterized by specific polar heads like glycosyl-inositol-phosphorylceramides (GIPC) and LCB like phytosphingosine (PHS, t18:1). Seeds also accumulate specific amino-derivate GIPC (NH<sub>2</sub>GIPC) (29).

92 Taken together, available data suggest that plant-derived SL can have beneficial effects on 93 the MetS onset. However, all previous studies were performed by using purified SL and, most often, plant SL were administered orally or intra-peritoneally, and not as diet 94 ingredient. The objective of the present study was therefore to assess (i) whether plant SL 95 provided as part of the diet (i.e. as a food ingredient in a food matrix) could counteract MetS 96 97 onset and (ii) whether SL acyl chain length is important in modulating the physiological and metabolic impact of dietary phytoSL. Because bioavailability is an essential prerequisite to 98 extra-digestive effects, digestibility of phytoSL with different acyl chain length had also to be 99 100 determined. Plant SL originating from two isogenic camelina cultivars in which SL were differing only by their fatty acid (FA) chain length were provided as seed meal to mice fed for 101 102 5 weeks with high-fat/high sucrose diets. The first outcome was to assess the bioavailability of dietary phytoSL at the digestive level, by using plant sphingolipidomic profiling, and their 103 104 possible impact on intestinal function. The second one was to evaluate if dietary phytoSL could slower the onset of the MetS, especially as regards dyslipidemia and IR. Finally, the 105 106 hypothesis that acyl chain length may modulate digestibility and bioactivity of phytoSL was 107 also tested for both the outcomes.

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#### 110 MATERIALS AND METHODS

Selection of C16-SL accumulating camelina line. Camelina sativa cultivar "Céline" was grown 111 in glasshouse conditions as described previously <sup>(30)</sup>. To generate plants accumulating SL rich 112 in C16 FA (C16-SL), the coding sequence of the Arabidopsis ceramide synthase AtLOH2 113 (At3g19260) was PCR amplified using LOH2EcoRI-F and LOH2XhoI-R primers modified with 114 115 EcoRI and XhoI restriction enzymes (LOH2EcoRI-F, GGAATTCATGGAATCGGTATCATCACG; LOH2XhoI-R, CCGCTCGAGCTAATCATCATCATCCTCTG) and cloned behind the soybean glycinin 116 promoter into the pBinGlyRed2 vectors after removal of the Luciferase coding sequence 117 using the EcoRI/XhoI enzymes. Plants were transformed by flower dip infiltration with 118 Agrobacterium tumefasciens carrying pBinGlyRed2::AtLOH2 and T1 seeds were selected 119 based on their red fluorescence <sup>(31)</sup>. A total of 107 T2 progeny were produced and screened 120 for their respective seed SL content by LC-MS/MS. Eight T3 progeny were eventually 121 selected and amplified and rescreened for their seed SL content. A homozygous line named 122 LOH2<sup>Y</sup> with the highest C16-SL content was finally selected and amplified as T4 and T5 123 generations. Complete SL profile was carried out on T5 seeds, as described below. 124

A total of 1476 wild type camelina and 1593 LOH2<sup>Y</sup> T4 plants were cultivated side by side 125 126 in the glasshouse, resulting in the production of 6.1 kg of Céline seeds (of which SL-FA are mostly C24) and 5.4 kg of T5 LOH2<sup>Y</sup> seeds (rich in C16-SL). Camelina seeds were crushed with 127 an Oilprinz KK8 F universal (Kern Kraft, Reut, Germany) at 60 rpm and with opening of 8 mm 128 to produce CamC24 and CamC16 meal and oil from Céline and LOH2<sup>Y</sup> seeds respectively. 129 Macronutriment composition of the camelina meal was determined as previously described 130 <sup>(32)</sup> and was (in weight %): 36.5/39.4 protein, 19.7/19.8 lipid, 29.4/28.8 fibre, 5.79/5.83 ashes 131 and 5.70/5.81 humidity for the CamC24 and CamC16, respectively. Fibre typology was the 132

<sup>133</sup> same in both meals (data not shown).

Animals and diets. Thirty-three 7-week old male C57BL/6JOlaHSD mice (Envigo, Gannat, 135 France) were housed in individual cages with wired floor, at  $22 \pm 1$ °C under 12-h light/dark 136 cycles (light on at 07:00 am) and acclimated to local conditions for 1 week. During the last 3 137 days of the week, 27 mice were progressively switched from the standard pelleted chow diet 138 139 (SAFE A04, Augy, France) to the control hypercaloric (HC-Con) diet (see below). At 8 weeks 140 of age, mice were divided into 4 groups and fed during 5 weeks with either the same chow 141 diet as before (normocaloric diet, NC group, n=6) or one of the three hypercaloric (HC) diets, two providing one of the two camelina meals (HC-CamC24 and HC-CamC16, n=9) and the 142 third one a control HC diet (HC-Con n=9) (Supplemental Table 1). Mice had free access to 143 food and tap water. Individual body weight was recorded weekly. The study was performed 144 according to the European directive for the use and care of laboratory animals 145 (2010/63/UE), and received the agreement of the local animal ethics committee and of the 146 ministerial committee for animal experimentation (Ref 20151I0317422745.v4-APAFIS#2701). 147 HC diets were prepared freshly twice a week, stored at 4°C, and provided daily to mice as 148 soft pellets. CamC24 and CamC16 meals from the commercial Céline cultivar and LOH2<sup>Y</sup> line 149 150 were incorporated into the HC diets (respectively HC-CamC24 and HC-CamC16). Camelina oil obtained from Céline seed crushing was kept at 4°C and incorporated into the HC-Con diet at 151 152 the same level as in the two HC-CamC24 and HC-CamC16 diets. It contains as previously described mostly polyunsaturated FA (53%) and monounsaturated FA (29%), as previously 153 described <sup>(31)</sup>. SL analysis of this oil showed the absence of NH<sub>2</sub>GIPC, the major SL in both 154 155 camelina meals.

156 Sphingolipid were extracted from camelina meals, oils and diets, as described previously 157 <sup>(29)</sup>. CamC24 and CamC16 meals contained the same levels (in nmol/g) of total phytoSL and 158 NH<sub>2</sub>GIPC, which was the major SL in both meals (92%) (Supplemental Table 2). In contrast, C24-NH<sub>2</sub>GIPC accounted for 82% of total NH<sub>2</sub>GIPC in CamC24, whereas C16-NH<sub>2</sub>GIPC 159 accounted for 90% of total NH<sub>2</sub>GIPC in CamC16. The amount of camelina meals to be 160 incorporated into the HC-CamC24 and HC-CamC16 diets (107g/kg) was calculated according 161 to the SL content of the camelina meals, the mice food intake (about 4 g/d for a HC diet) and 162 the amount of purified plant SL given to rodents in the literature (4-8 mg/d in mice, without 163 indication of their body weight) <sup>(14, 16)</sup>. SL profile of the HC-CamC24 and HC-CamC16 diets 164 165 reflected that of the respective meals, with NH<sub>2</sub>GIPC being the major SL with mostly by 24 carbons FA in CamC24 meal, and by 16 carbons FA in CamC16 meal (Table 1). As expected, 166 HC-Con diet contained very little NH<sub>2</sub>GIPC, whereas the NC diet was surprisingly rich in SL, 167 and especially in NH<sub>2</sub>GIPC, probably as a consequence of the high level of plant-based 168 169 materials (e.g. soya bean) in the commercial chow.

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*In vivo* measurements and sample collection. Food intake during 48 h was monitored after 6 d and 4 wk on the experimental diets. Feces were collected and weighed for further lipidomics at the end of the 48 h. Part of the feces was freeze-dried before assaying the dry matter content <sup>(32)</sup>. Apparent digestibility of the dry matter was calculated as follows: (intake-feces content)/intake\*100. SL apparent digestibility was calculated by applying the same formula to SL content measured in food and feces.

The body composition (fat mass and lean mass) was measured *in vivo* after 3 weeks on the experimental diets by dual energy X-ray absorptiometry (DEXA), using a Lunar PIXImus densitometer (GE Medical Systems, Buc, France). The stability of the device was controlled by a measurement of a phantom before each session. The mice were anesthetized by isoflurane inhalation during the measurement. Analysis of the images was performed with

the software provided with the device (Lunar PIXImus v2.10, GE Medical Systems, Buc,
 France), using auto-thresholding.

An oral glucose tolerance test (OGTT) was performed after 3 weeks on the experimental diets. Six hours fasted mice were weighed and a blood sample (~25  $\mu$ L) was taken from the tail vein ~15 min before (t0) gavage, then after 15, 30, 60, and 120 min following orally administration of a glucose solution (2 g/kg). Blood glucose concentration was immediately assayed using a standard glucometer (Onetouch, LifeScan, Boulogne-Billancourt, France). Blood was centrifuged (10 min, 1600 g, 4°C) and plasma stored at -20°C for subsequent assay of insulin.

After 5 weeks on the experimental diets, mice were fasted for 6 h. After a blood sample 191 has been taken from the tail vein for glucose determination, mice were deeply anesthetized 192 193 by isoflurane inhalation (IsoFlo, Axience, Pantin, France). Blood was taken by cardiac puncture before mice were euthanized by exsanguination. The abdominal cavity was then 194 opened and the following parts were removed and weighed: liver, epididymal adipose 195 196 tissue (EpAT), kidneys, spleen, small intestine, caecum and colon. The luminal contents of 197 the caecum and the colon were removed and weighed. Jejunum, colon and EpAT samples 198 were snap-frozen in liquid nitrogen and stored at -80°C. Blood samples were centrifuged as described above for plasma extraction and storage. 199

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*Ex vivo* intestinal measurements. To assess potential changes in intestinal barrier function, electrophysiological measurements were performed as previously described <sup>(33)</sup> with the following modifications: each mouse proximal colon was opened along the mesenteric line and mounted on an EasyMount Ussing chamber insert (Physiologic Instrument Inc, San Diego, CA, **USA**) with an exposed area of 0.2 cm<sup>2</sup>. Mounted tissues were left to equilibrate

their trans-epithelial potential ( $V_t$ , mV) 45 min before clamping them to 0 mV to obtain the short-circuit current ( $I_{sc}$ ,  $\mu A/cm^2$ ). The trans-epithelial electrical conductance ( $G_t$ , mS/cm<sup>2</sup>) was obtained using the Ohm law by the voltage deviation when the tissue was current pulsed with ± 5  $\mu A$ .

Paracellular permeability to fluorescein isothiocyanate (FITC)-dextran 4000 (FD4, Sigma) was determined during a 100-min period in Ussing chambers. Soon after tissue mounting ( $t_0$ ), FD4 was added to the chamber mucosal side at the final concentration of 0.250 mg/mL. Serosal samples were collected at  $t_0$  and after 100 min. To test for tissue viability at the end of the experiment, carbachol (CCh,  $10^{-4}$  M) was applied at the serosal side verifying the activation of the calcium-dependent chloride secretion by the  $I_{sc}$  increase.

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217 Biochemical analyses and physiological measurements. Blood glucose concentration was determined with an Accu-Chek® glucometer (Roche Diagnostics, Meylan, France). Plasma 218 triglyceride concentrations were determined by colorimetric enzymatic methods using a 219 220 commercial kit adapted for use in a 96-well microplate reader (Molecular Devices, Saint-Grégoire, France), and obtained from Randox (Roissy, France) (34, 35). Plasma insulin was 221 222 determined using enzyme-linked immuno-assay (Mouse insulin ELISA, Mercodia, Paris, France). Homeostasis model assessement of insulin resistance (HOMA-IR) index was 223 calculated using the formula: [fasting insulin (pmol/L) x fasting glucose (mmol/L)]/22.5. 224

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**Sphingolipidomics.** SL were extracted from feces, jejunum and colon as described previously  $^{(36)}$ . A volume of 500 µL isopropanol:hexane:water (55:20:25, v/v) and 10 µL standard mix [0.01 nmol of C12-Cer (d18:1-h12:0), 0.1 nmol of C12-GluCer (d18:1-h12:0) and 0.2 nmol of ganglioside (GM1)] were added to material and ground using a Polytron homogenizer. The 230 plunger was rinsed with 500 µL of extraction solvent and the sample incubated at 60°C for 15 min. After centrifugation at 4000 rpm for 5 min at room temperature, the supernatant 231 232 was recovered and the pellet extracted once more with 1 mL of solvent. Supernatants were combined and dried with a speed Vac. Then the samples were incubated at 50°C for 1 h with 233 234 500 μL of 33% methylamine solution in ethanol-water (7:3) and dried under nitrogen and 235 resuspended by sonication in 100 µL of tetrahydrofuran-methanol-water (2:1:2) containing 236 0.1% formic acid and filtrated prior to analysis by liquid chromatography coupled to mass 237 spectrometry (UPLC- MS/MS). UPLC- MS/MS analyses were carried out on a Waters Acquity UPLC system coupled to a Waters Xevo tandem guadrupole mass spectrometer equipped 238 with an ESI source (Waters, Milford, MA, USA). Chromatographic conditions, mass 239 240 spectrometric parameters, and multiple reaction monitoring (MRM) methods were defined as described previously <sup>(29)</sup>. 241

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Gene expression. Total RNA was extracted from EAT and colon samples using Trizol reagent 243 (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed with mouse-specific primers 244 245 (Eurogentec, Angers, France) to quantify mRNA level of the following genes : interleukin-6 246 (*II-6*), interleukin-1 $\beta$  (*II-16*) interleukin-10 (*II-10*) and tumor necrosis factor- $\alpha$  (*Tnf-\alpha*) in both 247 EAT and colon, plasminogen activator inhibitor-1 (Pai-1) and monocyte chemoattractant 248 protein-1 (*Mcp-1*) in EAT, and claudin 1 (*Cldn1*) and zonula occludens (*Tjp1*) in the colon. After cDNA synthesis from mRNA using High Capacity cDNA Reverse Transcription Kit 249 (ThermoFisher-Life Technologies, Les Ulis, France), Real-Time PCR was performed 250 251 subsequently on cDNA using the power SYBR Green PCR master mix and StepOne Real-Time PCR system (ThermoFisher-Life Technologies, Les Ulis, France). cDNA samples were assayed 252

in triplicate and gene expression levels for each sample were normalized relative to hypoxanthine-guanine phosphoribosyltransferase gene (*Hprt*) with  $2^{-\Delta\Delta Ct}$  calculation.

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Statistical analyses. Data are reported as means ± standard deviations. They were analysed
using the XLSTAT application (Addinsoft, Paris, France). Significance level was set at *P*<0.05.</li>
The effects of energy level (HC vs NC) and of camelina meal (HC-Con vs HC-CamC16/C24)
were tested using a 2-way ANOVA. When diet effect was significant, differences between
means were tested for significance using the *post-hoc* Tukey procedure.

261

262 **RESULTS.** 

Production of Camelina seeds with different sphingolipid contents. The high expression of 263 264 the C16 specific Ceramide synthase LOH2 from Arabidopsis in *Camelina sativa* led to seeds 265 with opposite sphingolipid profile (Supplemental Figure 1). Camelina seeds and meals (cake fraction from seeds) from commercial cultivar Céline (CamC24) presented characteristic 266 sphingolipid profile with high levels of NH2GIPC almost exclusively with very long chain FA 267 (C24). Conversely, the LOH2 expressing line LOH2<sup>Y</sup> (CamC16) accumulated almost exclusively 268 269 C16 NH<sub>2</sub>GIPC albeit the levels of total sphingolipids remained similar to CamC24. C16 GluCer 270 was also increased and C24 GluCer strongly decreased in CamC16 (Supplemental Table 2). A similar trend was also observed for Cer and hCer but at a much lower scale. The most 271 272 abundant LCB in seeds were t18:0 and t18:1 in both lines even if d18:1 and d18:0 were increased in  $LOH2^{\gamma}$  due to the accumulation of C16 NH<sub>2</sub>GIPC and GluCer .The accumulation 273 of C16 SL had a weak impact on plant fitness with a slight delay of germination and slower 274 early growth rate as well as a lower seed yield and weight for LOH2<sup>Y</sup> (data not shown). 275

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277 Body weight and associated parameters. During the 5 wk of MetS induction, all mice gained weight, but to a different extent (Figure 1). After 5 wk on the experimental diets, mice on 278 279 the HC-Con diet exhibited a higher weight gain (8.8±1.2g) than mice of the NC group, which gained 3.5±1.1 g only (Supplemental Table 3). In both HC-Cam groups, body weight gain, as 280 281 well as body weight after 2, 3 and 5 weeks on their respective diets, did not differ 282 significantly from the NC group, but were significantly lower than in the HC-Con group 283 (Figure 1 and Supplemental Table 3). Food intake (as kcal/d) tended to parallel body weight, 284 and was the highest in the HC-Con group after 6 d and 4 wk, whereas there was no difference between groups at both times when energy intake was adjusted for body weight 285 (Supplemental Table 3). Apparent digestibility (% intake of dry matter that was not found in 286 feces) was equivalent after 6 d or 4 wk on the same experimental diets. After 6 d, it was the 287 288 lowest in the NC group, intermediary in the HC-Con group and the highest in the HC-CamC24 and HC-CamC16 groups, whereas after 4 wk, it remained the highest in the HC-CamC24 289 group only (Supplemental Table 3). 290

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292 **Body composition.** After 3 wk on the experimental diets, the lean mass was the same in all groups, whereas the fat mass (absolute and relative to body weight) was as expected higher 293 in the three HC groups than in the NC group, without significant effect of the camelina meals 294 (Table 2). After 5 wk, mice of the HC-Con group were significantly heavier than those of the 295 296 NC group whereas mice of both HC-Cam groups showed intermediate body weight (Figure 1 297 and Table 2). Similarly, when compared to the NC group, adiposity (absolute and relative 298 weight of the EAT) was globally increased in the three HC groups, but to a lower extent in 299 the HC-Cam groups. While liver and small intestine weights were similar in all groups, the weights of the caecum and the colon, as well as of the caecum content, were significantlylower in the three HC groups.

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Blood and tissue markers associated with the MetS. During an OGTT after 3 weeks on the 303 experimental diets, glucose and insulin plasma concentration increased sharply during 15 304 min, then returned to fasting values after 120 min in all groups (Figure 2). When compared 305 306 to the NC group, the HC-Con group exhibited higher fasting glucose and insulin concentration (t0) and AUC over 120 min, as well as higher HOMA-IR index (Table 3 and 307 308 Figure 2). Besides, the increase in glucose concentration between t0 and t30 min, which 309 takes into account the difference in fasting glycemia between groups ( $\Delta$  glucose), was twofold higher in the HC-Con group than in the NC group. In both HC-Cam groups, fasting 310 glucose concentration and AUC over 120 min were similar to those of the HC-Con group, and 311 higher than in the NC-Con group, whereas insulin AUC and HOMA did not differ from the NC-312 Con group and were therefore lower than in the HC-group. Interestingly, insulin fasting 313 314 plasma concentration in HC-CamC16 mice did not differ from the NC mice and was significantly lower than in the HC-Con mice, which was not the case in mice HC-CamC24 315 mice. In the two HC-Cam groups,  $\Delta$  glucose between t30 and t0 of the OGTT was 316 317 intermediary between NC- and HC-Con groups.

At the end of the experiment, after 5 weeks on the experimental diets, fasting glucose concentrations did not differ from those after 3 weeks, remaining higher in the three HC groups than in the NC group (**Table 3**). Fasting triglyceridemia was identical in NC- and HC-Con groups, but was surprisingly 2-fold lower in the 2 HC-Cam groups. Compared to the NC-Con group, fasting cholesterolemia was higher in all the HC groups but only significantly in both HC-Cam groups.

324 Transcript levels of a few inflammatory and vascular markers were higher in mice fed the HC diets: *Mcp-1* and *Pai-1* in the adipose tissue (P = 0.011 and 0.002, respectively) and *II-1*  $\beta$ 325 326 in the colon (P = 0.039). However, according to the *post-hoc* Tukey test, and as compared to the NC group, *Mcp-1* and *II-1* $\beta$  **PRP PPPPP** were significantly higher in the HC-Con group 327 only, and *Pai-1* in the HC-Cam groups only (Table 4). Other mRNA levels of inflammatory 328 markers (II-6, II-16, II-10 and Tnf- $\alpha$  in the adipose tissue and II-6, II-10, Tnf- $\alpha$  in the colon) did 329 330 not differ between groups, neither did those of the tight junction protein encoding genes 331 Tjp1 and Cldn1 in the colon (data not shown).

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333 Colon epithelial barrier and permeability. Measurement of electrical parameters in Ussing chambers was performed on proximal colon (Table 5). There was no difference in the FD4 334 permeability within the diet groups, which indicated that paracellular pathway of the gut 335 336 barrier function in the colon was not altered (data not shown). The colon transepithelial 337 voltage  $(V_t)$ , which is usually negative at the colonocyte luminal side, was more negative in the 3 HC groups than in the control NC group, this effect being significantly less pronounced 338 in the HC-CamC16 group. Conversely, the short-circuit current  $(I_{sc})$  was higher in the three 339 HC groups, with no difference between them. Trans-epithelial conductance ( $G_t$ ) in the three 340 HC groups did not differ from that in the NC group, however the HC-CamC16 and the HC-341 342 Cam24 groups showed the highest and lowest values respectively.

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**SL intake, excretion and tissue profile.** Apparent NH<sub>2</sub>GIPC digestibility (**Table 6**) was calculated after 4 wk of experimental diets, using the *in vivo* measurement of food intake and fecal excretion and the analysis of complete plant sphingolipidomic profiles of diets (**Table 1**) and feces (data not shown). Since NH<sub>2</sub>GIPC accounted for nearly 80% of total SL in

348 HC-CamC16 and HC-Cam24 diets, intake and excretion were calculated in this fraction only. Mean individual NH<sub>2</sub>GIPC dietary intake, expressed as absolute amounts, grossly reflected 349 350 their dietary profile. It was very low in the HC-Con group, much higher in the NC group (over 40 fold increase), and even further increased in both HC-Cam groups by over 80 fold, with no 351 significant difference between the two groups. In contrast, NH<sub>2</sub>GIPC excretion differed 352 353 markedly between the HC-Cam groups. Excretion rate of NH<sub>2</sub>GIPC in the HC-CamC16 group 354 was 2.5-fold higher than that in the HC-CamC24 group. Consequentely, the apparent 355 digestibility of dietary NH<sub>2</sub>GIPC was significantly lower (80%) in the HC-CamC16 than in the three other groups (87-94%). 356

Concentration of the most abundant plant SL (NH<sub>2</sub>GIPC and GluCer with C16 and C24 FA) 357 was determined in the jejunum and colon walls (Table 7). Jejunum and colon of the NC 358 359 group contained similar amount of C24-NH2GIPC and C24-GluCer, while the latter was a minor component of the diet. Jejunum and colon of the HC-Con mice were nearly devoid of 360 phytoSL, as expected from their low level in the HC-Con diet. On the contrary, plant NH<sub>2</sub>GIPC 361 and GluCer were found in the intestine on mice fed with the camelina meals. As expected 362 C24-SL were the most prominent SL in the HC-CamC24 group, while C16-SL was the most 363 364 abundant in the HC-CamC16 group, with NH<sub>2</sub>GIPC being the most abundant SL in both groups. Tissue content of the various SL species was lower in the colon than in the jejunum, 365 366 however small levels of plant NH<sub>2</sub>GIPC were still present in the colon. Finally, the total concentration of SL (GluCer + NH<sub>2</sub>GIPC) in the intestine of HC-CamC16 mice was 367 approximately 2-fold higher in the jejunum (12.5 nmol/g) and in 5-fold higher in the colon 368 369 (2.14 nmol/g) than in HC-CamC24 mice (6.40 and 0.40 nmol/g in the jejunum and colon, 370 respectively). Interestingly, while C24-GluCer ratio between colon and jejunum was similar in 371 the two HC-Cam groups (0.38 vs 0.41), this ratio was much lower in the HC-Cam24 group

than in the HC-Cam16 group for C24-NH<sub>2</sub>GIPC (0.05 vs 0.26) and even lower in the HC-Cam16 group for C16-NH<sub>2</sub>GIPC (0.05 vs 0.16).

374

375 **DISCUSSION.** 

Digestive bioavailability of camelina SL. In most in vivo studies aiming to highlight the 376 377 mechanisms involved in potentially beneficial effects of plant SL at the systemic level, a 378 dramatic gap remains concerning the bioavailability of plant SL when provided in the diet. 379 Both animal and plant SL are hydrolysed into LCB, FA, and the polar head group in the small intestine, then eventually absorbed, reesterified, and secreted into the lymph (37-40). As 380 regards phytoSL, it is needed to document to what extent they are digested then absorbed, 381 in which form they are transported and made available to various tissues, and which 382 383 molecular species are stored in various body compartments.

To our knowledge, the present study quantifies, for the first time, intestinal digestibility 384 of a major phytoSL (NH<sub>2</sub>GIPC, accounting for nearly 80% of total SL in the HC-Cam diets) 385 386 when provided as a dietary component. Camelina NH<sub>2</sub>GIPC in the two HC-Cam diets exhibited the same LCB profile, and only differed in their FA length (mainly C24 in the 387 388 CamC24 meal and C16 in the CamC16 meal). Although apparent digestibility of dietary NH<sub>2</sub>GIPC was high, it was lower in the HC-CamC16 group, which could be accounted for by a 389 390 higher fecal excretion. However, the higher NH<sub>2</sub>GIPC content in the gut wall of the HC-Camc16 fed group, suggests some metabolic specificity during reesterification and excretion 391 into the lymph(Table 7). Besides, beyond this demonstration of the NH<sub>2</sub>GIPC availability at 392 393 the digestive level, NH<sub>2</sub>GIPC-containing SL, or their metabolites, have to be sought in other body compartments, this lack of information being a limitation of the present study. 394

395

396 Effects of the camelina meal on the early markers of the MetS. Plant SL have been shown to exert beneficial effects on some risk factors associated with the MetS, such as altered 397 glucose homeostasis, abnormal lipid profile, and low-grade chronic inflammation. In the 398 present study, we used our mouse model of the early steps of the MetS to explore the 399 effects of dietary plant SL provided by camelina meal in a hypercaloric diet <sup>(41)</sup>. As expected 400 401 from this model, after 5 wk on a hypercaloric diet rich in saturated FA and sucrose (HC-Con 402 group), mice exhibited (as compared to those on a normocaloric diet, NC-group) an increase 403 in body weight and adiposity (Table 2 and Figure 1), and in fasting glycemia (Table 4). After only 3 wk, these alterations were already detectable and were accompanied by higher 404 values of insulinemia and HOMA-IR index (Table 3), and of glucose and insulin postprandial 405 AUC (Figure 2). These findings are consistent with data obtained in mice fed a HC diet rich in 406 saturated FA<sup>(42)</sup>. 407

When SL was provided as camelina meal in the HC diet (HC-CamC24 and -C16 groups), 408 body weight gain and adiposity were reduced, but not the lean mass (Figure 1 and Table 2). 409 410 This specific effect of camelina meals on adiposity was not accounted for by a lower food 411 intake, since it was the same in all groups after 6 d and 4 wk when normalized for body 412 weight. Whether this effect is due to the presence of SL in the diet is questionable, since no significant effect of phytoSL was reported for body weight, fat mass or body mass index in 413 previous studies <sup>(14-17, 43, 44)</sup>. In our study, the presence of other components of the camelina 414 415 meal, such as fibers, may be responsible for a lower nutrient absorption, and subsequent 416 bioavailability, lowering body storage of lipids, either exogenous (dietary) or endogenous (de 417 novo synthesised). However, a specific effect of phytoSL may not be precluded, since a very 418 recent study showed that mice fed a high-fat diet supplemented with milk polar lipids containing 25% SL gained less weight than mice on the high-fat diet alone <sup>(45)</sup>. 419

420 In parallel, in mice fed by either camelina meal, fasting insulinemia and HOMA-IR index were normalized, as well as insulinemia AUC during an OGTT, and triglyceridemia decreased 421 (Table 3 and Figure 2). These findings are grossly consistent with previous studies using 422 purified SL or LCB in rodent mice models exhibiting features of the MetS or in human 423 subjects with a MetS. Indeed, various SL or LCB decreased triglyceridemia in mice or sand 424 rats on a hypercaloric diet <sup>(14, 20)</sup> whereas phytosphingosine decreased glycemia in subjects 425 with a MetS<sup>(15)</sup> and improved glucose homeostasis in human subjects with a MetS and 426 various rodent models, <sup>(15-19)</sup>. In contrast, the present study showed no effect on 427 cholesterolemia, which is inconsistent with previous studies that repeatedly reported a 428 decrease in plasma total, LDL, or non-HDL cholesterol in various rodent models and in 429 subjects with a MetS<sup>(14, 15, 17, 20)</sup>. One originality of our short-term study is the demonstration 430 that, when focusing on the onset of the MetS, an early alteration of glucose homeostasis, as 431 demonstrated by insulin resistance occurrence both at the fasting state and during an OGTT, 432 may be counteracted by a phytoSL-rich matrix after only 3 weeks on the experimental diets. 433 434 The mechanisms by which dietary SL may exert systemic effects remain largely speculative. First, in response to various SL or LCB, a decrease in the postprandial absorption 435 436 of dietary triglycerides and cholesterol has been suggested, and would explain the downstream effects on lipid metabolism at the hepatic or plasma level <sup>(14, 43)</sup>. Second, gene 437 regulation by SL or LCB, including activation of various PPARs, and resulting in beneficial 438 effects on insulin sensitivity, glucose tolerance and lipid metabolism have been also 439 demonstrated <sup>(17, 20, 43)</sup>. Finally, it has been recently shown that animal SL may affect gut 440 microbiota in mice in a way that would limit some detrimental effects of HF diets (45, 46). All 441 these mechanisms, which remain to be investigated in our model, would contribute to lower 442 adiposity and slower insulin resistance in mice fed the HC-Cam diets (Table 2). 443

444 In response to a diet rich in saturated FA and sucrose, the increased adipose tissue often features an inflammatory phenotype <sup>(4)</sup>. In the present study that targeted the initial steps of 445 the MetS, there was no evidence that gene expression of pro-inflammatory cytokines was 446 enhanced in the adipose tissue, despite an increased adiposity, and providing SL-rich 447 camelina meal had no significant effect. Gene expression of two others inflammatory 448 449 markers, Mcp-1 and Pai-1, was enhanced in response to the HC diet (HC-Con group). 450 Attenuation of Mcp-1 mRNA level may be related to the lower adiposity of mice on both HC-451 Cam diets, whereas the reason why the HC-Cam diets aggravated the mRNA level of Pai-1, remains to be investigated. 452

To our knowledge, the present study, provides the first evidence that a mixture of natural plant SL, essentially in the form of NH<sub>2</sub>GIPC, and provided in their original matrix, exerted some beneficial effects during the MetS onset. However, the role of other camelina meal components, such as phospholipids, polyphenols or specific fibers cannot be excluded <sup>(47, 48)</sup>.

Effects of the camelina meal on the intestine wall. When compared to the NC group, the 458 HC-Con diet enhanced a few inflammation markers in the colon and the adipose tissue 459 460 (Table 4). In contrast, in mice fed the camelina meals, mRNA levels of Mcp-1 in the adipose tissue and of *II-16* in the colon did not differ from those in the NC group (**Table 4**). The role 461 462 of SL in inflammatory processes is still in debate, but a few studies support the hypothesis that dietary phytoSL (as maize GluCer) or animal SL (as sphingomyelin) alleviate gut 463 inflammation in rodent models <sup>(23-28)</sup>. How plant SL may improve intestinal inflammation 464 465 remains nonetheless highly speculative. A study using Caco-2 cells suggested that SL may 466 interact with epithelial cells without being absorbed. In these in vitro conditions, plant 467 GluCer remained at the cell surface, but decreased LPS-induced production of inflammatory 468 cytokines, maybe by affecting the interactions between LPS and its TLR at the cell surface <sup>(28)</sup>. 469 Similar interactions with non-digested plant SL or their metabolites may contribute *in vivo* to 470 modulate the inflammatory status of the intestine wall. The intestinal microbiota is likely to 471 contribute to these interactions, since it is able to metabolize animal SL, which would 472 contribute to regulate cytokine production <sup>(24, 49)</sup>.

473 Interestingly, epithelial integrity of the colon was specifically modified by the HC-CamC16 474 diet, as demonstrated by its effects on transepithelial voltage ( $V_t$ ) and epithelial conductance  $(G_t)$  (**Table 5**).  $V_t$  describes the unidirectional transport of sodium from the luminal to the 475 serosal side of the epithelium. This potential difference is usually negative at the colonocyte 476 luminal side, and was even more negative with HC diets. This effect of HC-diets reflects an 477 increase in the driving force of the colonocytes to allow the sodium, chloride and water 478 479 absorption from the colon lumen, and therefore a loss of permeability. This decrease in  $V_t$ may be related to the high fat content of the diet, known to alter electrical parameters of 480 transcellular barrier function (42, 50, 51). This damage was attenuated in mice given the diet 481 482 containing camelina SL rich in C16 FA (HC-CamC16).  $V_t$  increase in HC-Camc16 mice was also associated with higher epithelial conductance  $(G_t)$  which was not due to a loss of epithelial 483 484 permeability, at least via the paracellular pathway, because it was not accompanied by an increase in the apical-to-basal flux of the paracellular marker FD4 in any group. The absence 485 486 of dietary effects on the gene expression of two tight junction proteins, claudin 1 (Cldn1) and zonula occludens (*Tjp1*), confirms that the paracellular permeability was not affected by any 487 of the HC diets. Rather, we suggest that, in the colon of the HC-CamC16 mice, this increased 488 489  $G_t$  may reflect a compensatory mechanism that counteracts the increase of actively 490 transported ions (as evidenced by the higher short-circuit current  $-I_{c}$  in all HC groups) by increasing passive movement of ions. This would explain the lower value of  $V_t$  in the HC-491

492 CamC16 group. Nevertheless, further investigations would be needed to determine the precise mechanisms involved in the action of camelina SL (native or digested products) on 493 activity and/or expression of colon transporters and exchangers that ensured colon barrier 494 integrity and flux. Yet, it can be concluded from the present study than differences in the FA 495 profile of dietary plant SL, and particularly the enrichment of C16 FA in the experimental 496  $LOH2^{\gamma}$  line (to the detriment of C24 FA in the commercial Céline cultivar) modulate the 497 deleterious effects of a high-fat diet on colon epithelial barrier function, and more 498 specifically on transepithelial permeability. 499

500

In conclusion, phytoSL provided in a plant matrix delays the onset of the MetS, by 501 502 reducing adiposity and insulin resistance. Involved mechanisms at the intestinal and systemic levels remain mostly speculative at this initial stage, but it may be hypothesized 503 that some of the effects observed, including on the adipose tissue and blood parameters, 504 results from an impact of phytoSL or their metabolites at the intestinal level. Moreover, 505 506 some of these effects are modulated by the FA length of the SL, since they are sometimes more pronounced in presence of C16-NH<sub>2</sub>GIPC. More researchs are required to uncover the 507 508 importance of FA length in the interactions between phytoSL, the intestinal wall, and systemic effects. In this context, interactions of phytoSL and their metabolites with the 509 510 microbiota may be a promising avenue of investigation.

511

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525

#### 526 Supporting information.

- 527 Supplemental Table 1. Composition and nutritional values of the experimental diets.
- 528 Supplemental Table 2. SL composition of the camelina meals (nmol/g)
- 529 Supplemental Table 3. Body weight gain and food intake
- 530 Supplemental Figure 1. NH<sub>2</sub>GIPCs and GICers profiles in Camelina sativa seeds from the
- 531 commercial Céline cultivar (CamC24) and from the LOH2<sup>Y</sup> line (CamC16).

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688		NC	HC-Con	HC-CamC24	HC-CamC16	
689	Total Cer	125	54	61	70	
690	C16-Cer	11.4	8.36	9.39	14.7	
604	C24-Cer	43.7	14.3	16.6	17.5	
691	Total hCer	107	34	44	53	
692	C16-hCer	4.99	5.61	5.84	10.9	
693	C24-hCer	45.2	13.0	18.1	19.7	
	Total GluCer	20	0.44	10.6	7.6	
694	C16-GluCer	6.26	0.14	1.53	4.3	
695	C24-GluCer	8.30	0.20	7.08	2.4	
696	Total NH <sub>2</sub> GIPC	222	5.31	444	463	
co <b>-</b>	C16-NH <sub>2</sub> GIPC	0.82	0.38	6.71	421	
697	C24-NH <sub>2</sub> GIPC	98.9	2.61	363	33	
698	Total SL	473	94.0	560	593	
699	C16-SL	23.4	14.3	23.5	450	
700	C24-SL	194	30.0	404	72.6	
700	Total NH <sub>2</sub> GIPC	16.9	5.6	70.3	70.4	
701	(% of total SL)	40.9	5.0	79.5	70.1	
702	C16-NH <sub>2</sub> GIPC	0 37	7 16	1 5 1	00 0	
702	(% of total $NH_2GIPC$ )	0.57	7.10	1.51	90.9	
703	C24-NH <sub>2</sub> GIPC	11 5	40.2	01 0	7 1	
704	(% of total $NH_2GIPC$ )	44.5	49.2	01.0	7.1	

687 Table 1. SL composition of the experimental diets (nmol/g).

NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the experimental LOH2 cultivar rich in C16 fatty acids); hCer, hydroxyceramide; GluCer, glucosylceramide; NH<sub>2</sub>GIPC, aminoglycosyl-inositol-phosphoryl-ceramide.

	NC	HC-Con	HC-CamC24	HC-CamC16	<i>P</i> ANOVA
After 3 weeks					
Body weight (fed state, g)	25.6±2.0 <sup>ª</sup>	29.9±2.4 <sup>b</sup>	27.1±1.4 <sup>ª</sup>	27.0±1.0 <sup>ª</sup>	0.0013
Lean body mass (g)	21.2±1.5 <sup>ª</sup>	21.0±0.9 <sup>a</sup>	20.0±1.1 <sup>ª</sup>	20.5±0.7 <sup>a</sup>	0.1086
Fat body mass (g)	4.28±0.79 <sup>a</sup>	8.31±1.92 <sup>c</sup>	6.69±1.72 <sup>bc</sup>	6.19±1.07 <sup>ab</sup>	0.0006
Fat body mass (% body weight)	16.7±2.4 <sup>ª</sup>	28.1±4.8 <sup>b</sup>	24.9±5.2 <sup>b</sup>	23.1±3.2 <sup>b</sup>	0.0004
After 5 weeks					
Body weight (fasted state, g)	26.7±2.5 <sup>ª</sup>	31.5±4.1 <sup>b</sup>	28.6±0.5 <sup>ab</sup>	28.6±1.3 <sup>ab</sup>	0.0148
EAT (mg)	558±117 <sup>ª</sup>	1446±520 <sup>b</sup>	911±199 <sup>a</sup>	946±186 <sup>a</sup>	0.0003
EAT (% body weight)	2.08±0.36 <sup>a</sup>	4.48±1.18 <sup>c</sup>	3.19±0.60 <sup>b</sup>	3.29±0.52 <sup>b</sup>	0.0002
Liver (g)	1.18±0.15 <sup>ª</sup>	1.16±0.16 <sup>ª</sup>	1.07±0.20 <sup>ª</sup>	1.05±0.17 <sup>ª</sup>	0.4780
Small intestine (mg)	947±96 <sup>ª</sup>	836±91 <sup>ª</sup>	830±60 <sup>a</sup>	862±84 <sup>a</sup>	0.0546
Caecum (mg)	109±23 <sup>ª</sup>	58±33 <sup>b</sup>	69±18 <sup>b</sup>	67±13 <sup>b</sup>	0.0002
Caecum content (mg)	301±76 <sup>ª</sup>	157±41 <sup>b</sup>	164±53 <sup>b</sup>	185±31 <sup>b</sup>	<0.0001
Colon (mg)	193±19 <sup>ª</sup>	139±25 <sup>b</sup>	132±15 <sup>b</sup>	131±29 <sup>b</sup>	<0.0001
Colon content (mg)	115±17 <sup>a</sup>	79±21 <sup>ª</sup>	91±28 <sup>ª</sup>	101±34 <sup>a</sup>	0.0968

#### 711 Table 2. Body composition after 3 and 5 weeks on the experimental diets

Values are means  $\pm$  standard deviations for 6 and 9 mice in the NC and the three HC- groups, respectively. Mean values within a row, not sharing a same superscript letter, were significantly different at *P*<0.05.

NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the experimental LOH2 cultivar rich in C16 fatty acids); EAT, epididymal adipose tissue. 718 Table 3. Blood markers associated with a MetS after 3 weeks (during an OGTT) and 5

	NC	HC-Con	HC-CamC24	HC-CamC16	<i>P</i> ANOVA
3 weeks					
Glucose t0 (g/L)	1.22±0.10 <sup>a</sup>	1.59±0.13 <sup>b</sup>	1.52±0.14 <sup>b</sup>	1.51±0.13 <sup>b</sup>	<0.0001
Insulin t0 (µg/L)	0.67±0.33 <sup>a</sup>	1.37±0.38 <sup>b</sup>	0.90±0.42 <sup>ab</sup>	0.78±0.33 <sup>ª</sup>	0.0035
HOMA-IR index	5.63±2.48 <sup>a</sup>	15.60±5.03 <sup>b</sup>	9.73±4.36 <sup>ª</sup>	8.33±3.54 <sup>a</sup>	0.0004
∆ glucose (t30-t0 g/L)	0.59±0.17 <sup>ª</sup>	1.41±0.50 <sup>b</sup>	1.22±0.78 <sup>ab</sup>	0.91±0.32 <sup>ab</sup>	0.0261
5 weeks					
Glucose (g/L)	1.18±0.17 <sup>ª</sup>	1.60±0.17 <sup>b</sup>	1.58±0.14 <sup>b</sup>	1.55±0.14 <sup>b</sup>	<0.0001
Triglyceride (g/L)	0.69±0.18 <sup>ª</sup>	$0.60 \pm 10^{a}$	0.35±0.04 <sup>b</sup>	0.38±0.09 <sup>b</sup>	<0.0001
Cholesterol (g/L)	1.01±0.18 <sup>ª</sup>	1.55±0.47 <sup>ab</sup>	1.73±0.32 <sup>b</sup>	1.74±0.19 <sup>b</sup>	0.0088

719 weeks on the experimental diets.

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Values are means  $\pm$  standard deviations for 6 and 9 mice in the NC and the three HC- groups, respectively. Mean values within a row, not sharing a same superscript letter, were significantly different at *P*<0.05.

NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the

commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the

726 experimental LOH2 cultivar rich in C16 fatty acids).

727 HOMA-IR: homeostasis model assessment of insulin resistance

729 Table 4. Relative gene expression level of some markers of low-grade inflammation.

	NC	HC-Con	HC-CamC24	HC-CamC16	<i>P</i> ANOVA
EAT					
Мср-1	1.19±0.72 <sup>ª</sup>	3.55±2.07 <sup>b</sup>	2.79±1.10 <sup>ab</sup>	2.08±0.77 <sup>ab</sup>	0.0168
Pai-1	1.24±0.81 <sup>ª</sup>	2.67±1.02 <sup>ab</sup>	4.12±1.64 <sup>b</sup>	3.17±1.28 <sup>b</sup>	0.0021
<u>Colon</u>					
II-16	1.03±0.25 <sup>ª</sup>	2.03±1.02 <sup>b</sup>	1.60±0.39 <sup>ab</sup>	1.58±0.44 <sup>ab</sup>	0.0707

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Values are means  $\pm$  standard deviations for 6 and 8-9 mice in the NC and the three HCgroups, respectively. They were calculated according to the 2<sup>- $\Delta\Delta$ Ct</sup>, where  $\Delta$ Ct = (Ct target gene - Ct HPRT) normalized to the NC group. Mean values within a row, not sharing a same superscript letter, were significantly different at *P*<0.05.

NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the experimental LOH2 cultivar rich in C16 fatty acids).

740 Table 5. Electrical measurement of colon epithelium parameters in Ussing chambers.

	NC	HC-Con	HC-CamC24	HC-CamC16	<i>P</i> ANOVA
$V_t$ (mV)	-2.78±0.15 <sup>ª</sup>	-8.45±0.83 <sup>b</sup>	-9.08±1.32 <sup>b</sup>	-5.90±1.30 <sup>c</sup>	<0.0001
<i>I<sub>sc</sub></i> (μA/cm <sup>2</sup> )	59±10 <sup>°</sup>	166±17 <sup>b</sup>	149±14 <sup>b</sup>	142±3 <sup>b</sup>	<0.0001
$G_t$ (mS/cm <sup>2</sup> )	16.8±2.2 <sup>ab</sup>	16.5±1.8 <sup>ab</sup>	14.0±2.4 <sup>a</sup>	20.1± 3.3 <sup>b</sup>	0.0396

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Measures were acquired after 45-minutes epithelium equilibration in recording buffer.  $V_{t}$ 743 trans-epithelial voltage;  $I_{sc}$  short-circuit current;  $G_{b}$  trans-epithelial electrical conductance. 744 Values deviations for 3-4 745 are means ± standard mice in each group. Mean values within a row, not sharing a same superscript letter, were significantly different 746 747 at *P*<0.05. NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the 748 commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the 749

750 experimental LOH2 cultivar rich in C16 fatty acids).

Table 6. Intake, excretion, and apparent digestibility of NH<sub>2</sub>GIPC after 4 weeks on the
 experimental diets.

	NC	HC-Con	HC-CamC24	HC-CamC16	<i>P</i> ANOVA
NH <sub>2</sub> GIPC Intake	784±36 <sup>b</sup>	17.7+1.5 <sup>c</sup>	1209+186 <sup>°</sup>	1358+195 <sup>ª</sup>	<0.0001
(nmol/d)					
NH <sub>2-</sub> GIPC Excretion	43 7+10 5 <sup>c</sup>	2 45+0 84 <sup>c</sup>	113+33 <sup>b</sup>	278+48 <sup>a</sup>	<0.0001
(nmol/d)	45.7 ±10.5	2.45±0.04	115-55	270140	0.0001
NH <sub>2</sub> GIPC Excretion	5 7/1+1 //9 <sup>C</sup>	13 73+4 30 <sup>b</sup>	9 /0+2 58 <sup>0</sup>	20.33±2.61 <sup>ª</sup>	<0.0001
(intake %)	5.74±1.45	13.7314.30	J.40±2.50		
Apparent digestibility	9/ 3+1 5 <sup>a</sup>	86 3+2 6 <sup>b</sup>	90 6+2 6 <sup>ab</sup>	79 7+2 6 <sup>c</sup>	<0.0001
(intake %)	54.5±1.5	00.3±2.0	50.0+2.0	75.7±2.0	<b>\0.0001</b>

Values are means ± standard deviations for 6 and 9 mice in the NC and the three HC- groups,

755 respectively. Mean values within a row, not sharing a same superscript letter, were

r56 significantly different at *P*<0.05.

NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the
commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the
experimental LOH2 cultivar rich in C16 fatty acids).

761 **Table 7. Sphingolipid levels in the jejunum and colon (nmol/g).** 

	NC	HC-Con	HC-CamC24	HC-CamC16	<i>P</i> ANOVA
Jejunum					
C16 GluCer	$0.025 \pm 0.021^{ab}$	nd	$0.009 \pm 0.008^{b}$	$0.027 \pm 0.016^{a}$	0.0004
C24-GluCer	0.499 ± 0.295 <sup>a</sup>	$0.108 \pm 0.040^{b}$	$0.333 \pm 0.150^{a}$	$0.289 \pm 0.108^{ab}$	0.0006
C16-NH₂GIPC	nd	nd	$0.248 \pm 0.122^{b}$	11.683 ± 2.085 <sup>a</sup>	<0.0001
C24-NH <sub>2</sub> GIPC	$0.671 \pm 0.231^{b}$	nd	5.799 ± 1.609 <sup>a</sup>	$0.481 \pm 0.138^{b}$	<0.0001
Colon					
C16-GluCer	nd	nd	nd	nd	-
C24-GluCer	$0.151 \pm 0.028^{a}$	$0.081 \pm 0.044^{a}$	$0.125 \pm 0.036^{a}$	$0.118 \pm 0.069^{a}$	0.0661
C16-NH₂GIPC	nd	nd	nd	1.901 ± 0.625	<0.0001
C24-NH <sub>2</sub> GIPC	0.382 ± 0.214 <sup>a</sup>	nd	$0.279 \pm 0.072^{a}$	0.123 ± 0.056 <sup>b</sup>	<0.0001

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Values are means  $\pm$  standard deviations for 6 and 9 mice in the NC and the three HC- groups, respectively. Mean values within a row, not sharing a same superscript letter, were significantly different at *P*<0.05; nd, not dectected.

NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the
 commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the
 experimental LOH2 cultivar rich in C16 fatty acids).





773 experimental diets.



Figure 2. Plasma kinetics of glucose and insulin during an OGTT after 3 weeks on the
 experimental diets.

783 Legend to Figures.

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#### 785 Figure 1. Body weight curve.

Body weight (g) was measured longitudinally in the fed state for 37d. At d0, after they were acclimated to local conditions for 1 week, mice that have been given progressively the HC-Con diet during this 1<sup>st</sup> week, were switched to the HC-Con, HC-CamC24 and HC-CamC16 according to their experimental group.

Values are means ± standard deviations for 6 and 9 mice in the NC and the three HC- groups,
respectively.

NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the experimental LOH2 cultivar rich in C16 fatty acids).

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Figure 2. Plasma kinetics of glucose and insulin during an OGTT after 3 weeks on the
 experimental diets.

Glycemia (Panel A) and insulinemia (Panel B) have been measured for 120 min and their respective area under the curve (AUC) were calculated over the same period of time (Panels B and D). Values are means  $\pm$  standard deviations for 6 and 9 mice in the NC and the three HC- groups, respectively. Mean AUC values not sharing a same superscript letter, were significantly different at *P*<0.05.

804	NC, normo-caloric; HC, hyper-caloric; Con, control; CamC24, camelina meal C24 (from the
805	commercial Céline cultivar rich in C24 fatty acids); CamC16, camelina meal C16 (from the
806	experimental LOH2 cultivar rich in C16 fatty acids).
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