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## **Adverse Effects of Oseltamivir Phosphate Therapy on the Liver of LDLR 2/2 Mice Without Any Benefit on Atherosclerosis and Thrombosis**

Olivier Bocquet, Amandine Wahart, Thomas Sarazin, Elise Vincent, Christophe Schneider, Anne Fougerat, Stéphanie Gayral, Aubéri Henry, Sébastien Blaise, Béatrice Romier-Crouzet, et al.

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1        **Adverse effects of oseltamivir phosphate therapy on the liver of LDLR<sup>-/-</sup>**  
2                    **mice without any benefit on atherosclerosis and thrombosis**

3

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26 **Abstract**

27 Desialylation, governed by sialidases or neuraminidases, is strongly implicated in a wide  
28 range of human disorders, and accumulative data show that inhibition of neuraminidases, such  
29 as NEU1 sialidase, may be useful for managing atherosclerosis. Several studies have reported  
30 promising effects of oseltamivir phosphate, a widely used anti-influenza sialidase inhibitor,  
31 on human cancer cells, inflammation and insulin resistance. In this study, we evaluated the  
32 effects of oseltamivir phosphate on atherosclerosis and thrombosis and potential liver toxicity  
33 in LDLR<sup>-/-</sup> mice fed with high fat diet. Our results showed that oseltamivir phosphate  
34 significantly decreased plasma levels of LDL-cholesterol and elastin fragmentation in aorta.  
35 However, no effect was observed on both atherosclerotic plaque size in aortic roots and  
36 chemically-induced thrombosis in carotid arteries. Importantly, oseltamivir phosphate  
37 administration had adverse effects on the liver of mice and significantly increased mRNA  
38 expression levels of F4/80, IL-1 $\beta$ , TGF- $\beta$ 1, MMP-12 and collagen. Taken together, our  
39 findings suggest that oseltamivir phosphate has limited benefits on atherosclerosis and carotid  
40 thrombosis and may lead to side effects in the liver with increased inflammation and fibrosis.

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## 51 **Introduction**

52 Human neuraminidases, or sialidases, are glycoside hydrolases which release terminal sialic  
53 acid residues from glycoproteins, glycolipids and oligosaccharides (1). Four types of  
54 mammalian neuraminidases (NEU), encoded by different genes, have been described with  
55 distinct substrate specificity and subcellular localization: NEU1 (2), NEU2 (3), NEU3 (4) and  
56 NEU4 (5). Sialidases have been implicated in a wide range of human disorders, including  
57 cancers, metabolic and cardiovascular diseases (6). Accumulative data highlighted sialidases  
58 as promising therapeutic targets, and modulating the activity of these enzymes may have  
59 beneficial effects in several pathologies.

60 Due to the lack of selective inhibitors, the vast majority of studies reported so far have used  
61 DANA (2,3- dehydro-2-deoxy-N-acetylneuraminic acid), a non-selective inhibitor of the four  
62 NEU isoenzymes, or oseltamivir phosphate (OP), an anti-influenza drug (7). OP is a prodrug  
63 which is converted by carboxyl esterase enzymes into the active oseltamivir carboxylate. OP  
64 is a sialic acid analogue which interacts with and blocks sialidase enzymes of the influenza  
65 virus, blocking their ability to release virions in the infected cells (8). Although OP  
66 demonstrates almost no appreciable inhibition of human sialidases (9, 10), contradictory  
67 results were obtained with this drug when tested on human cancer cells using both *in vitro* and  
68 *in vivo* models (11-15). It was also reported that OP reverses the epithelial to mesenchymal  
69 transition process and increases drug sensitivity of chemoresistant human cancer cells (16,  
70 17). Moreover, in human monocytic cells, OP has been shown to decrease pro-inflammatory  
71 cytokines production in response to LPS (18, 19) or thymoquinone, derived from the  
72 nutraceutical black cumin oil (20). Both agonists activate toll-like receptors and trigger NF $\kappa$ B  
73 activation through NEU1 and NEU4, respectively. Finally, reported effects of OP on NEU1-  
74 mediated regulation of insulin receptor (IR), phosphorylation of IR $\beta$  and insulin receptor  
75 substrate-1 (IRS-1) in response to insulin (21) and on IR transactivation by G-protein coupled

76 receptors (22), suggest potential application of OP treatment in insulin resistance and type 2  
77 diabetes.

78 Accumulative evidences also showed that neuraminidases inhibition, and more precisely  
79 inhibition of NEU1 sialidase, may be useful for managing atherosclerosis development (23-  
80 25). Atherosclerosis induces the progressive luminal narrowing of arteries, which is the  
81 underlying cause of myocardial infarction and ischemic stroke. Formation of atherosclerotic  
82 plaques involve the accumulation of cholesterol and inflammatory cells in the sub-endothelial  
83 space of the vessel intima and is closely linked to extracellular matrix remodeling and elastic  
84 fibers degradation (26). However, evaluation of OP efficacy in animal models of  
85 atherosclerosis has never been reported so far. In addition, the use of OP in patients has been  
86 associated with adverse effects such as liver toxicity (9, 27-29), and the benefits versus harms  
87 of OP remains to be further evaluated before potential application in human for treatment of  
88 cancer, metabolic and cardiovascular diseases.

89 In the present paper, we evaluated the effects of OP administration on atherosclerosis and  
90 chemically-induced arterial thrombosis in LDLR<sup>-/-</sup> mice fed with a high fat diet (HFD). We  
91 assessed the effects of OP on plasma lipid parameters, elastin fragmentation in aorta, and liver  
92 inflammation and fibrosis. Whereas OP was shown to significantly reduce plasma LDL-  
93 cholesterol levels and elastic fibers fragmentation in aorta, no benefit was observed on  
94 atherosclerosis development after 12 weeks of HFD, and on the time for complete carotid  
95 artery occlusion following FeCl<sub>3</sub> injury. Importantly, adverse effects were observed in the  
96 liver of mice with increased inflammation and fibrosis. In conclusion, this study suggests that  
97 OP has limited effects on atherosclerosis and carotid thrombosis and that caution must be  
98 taken regarding its **potential** adverse effects on the liver.

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100

## 101 **Materials and methods**

### 102 **Animals**

103 All mouse experiments were approved by the Ethics Committee for Animal Welfare of the  
104 University of Reims Champagne-Ardenne (CEEA-RCA no 56) and the French Ministry of  
105 Research (APAFIS # 5464-2016052416307263v4) being compliant with the European  
106 Directive 2010/63/UE. LDLR<sup>-/-</sup> (B6.129S7-Ldlr<sup>tm1Her</sup>/J) female mice were purchased from  
107 Charles River Laboratories (L'Arbresle, France). LDLR<sup>-/-</sup> mice (12 weeks-old) were fed for  
108 12 weeks with HFD + 0.15% cholesterol or normal diet (ND) (Special Diet Services,  
109 DIETEX France Sarl, Argenteuil, France). The HFD was constituted by crude protein 22.6%  
110 (w/w), crude fat 23.0% (w/w), crude fibers 4.6% (w/w), Ash 4.2% (w/w), carbohydrate 39.8%  
111 (w/w).

112

### 113 **Oseltamivir phosphate**

114 OP capsules (75 mg, Roche) were reconstituted in sterile phosphate-buffered saline (PBS),  
115 and centrifuged at 1,000 rpm for 10 minutes to obtain OP in the supernatant as previously  
116 reported (13). The stock-extracted OP solution had a concentration of 15 mg/mL. OP was  
117 used at a concentration (50 mg/kg) that completely ablates tumor vascularization, tumor  
118 growth and spread to the lungs in a mouse model of heterotopic xenografts of MDA-MB-231  
119 tumors (13) and twice lower than the one used by Gilmour et al in a mouse model of  
120 heterotopic xenografts of human pancreatic cancer (12). OP or vehicle were intraperitoneally  
121 injected each week, starting at the onset of HFD, and for 12 weeks.

122

### 123 **Intraperitoneal glucose tolerance test**

124 Glucose tolerance tests were performed according to Heikkinen *et al* (30). Briefly, 20%  
125 aqueous glucose solution was administrated by intraperitoneal injection (2 mg glucose/g body

126 weight) in mice fasted for 4 h. Glycemia was then measured from a blood drop taken from the  
127 tail of mice at baseline and 15, 30, 45, 60 and 90 min after glucose administration.

128

### 129 **Blood measurements**

130 At diet completion, mice were fasted for 6 h and blood was collected from the retro-orbital  
131 sinus into heparin tubes, centrifuged and plasma aliquots were kept at -80°C. Plasma glucose,  
132 insulin, total-, LDL-, and HDL-cholesterol, triglyceride and free fatty acids levels were  
133 measured by the Institut Clinique de la Souris (Ilkirch, France). Homeostatic model  
134 assessment (HOMA) for insulin resistance (HOMA-IR) was calculated according to (30):  
135  $HOMA-IR = (FPI \times FPG) / 22.5$  where FPI was the fasting plasma insulin concentration  
136 ( $\mu\text{U/mL}$ ) and FPG was the fasting plasma glucose concentration ( $\text{mmol/L}$ ). Glycated  
137 haemoglobin ( $\text{HbA}_{1c}$ ) was quantified by immunoassay using the DCA Vantage Analyzer  
138 (Siemens Healthcare, Erlangen, Germany).

139

### 140 **Quantification of elastin fragmentation in the aorta**

141 Mice were sacrificed by cervical dislocation, and aorta were collected rapidly and embedded  
142 within optimal cutting temperature (OCT) (Tissue-Tek) compound before placing them at -  
143 80°C until analysis. Cryosections (10  $\mu\text{m}$ ) from aorta was visualized under an epifluorescence  
144 microscope (Olympus BX51WIF, Olympus France S.A.S., Rungis, France) connected to a  
145 digital camera (Olympus CAM-UC90). Elastin has autofluorescent properties and emits  
146 fluorescence at 500- 560 nm when excited at 488 nm (31).

147

### 148 **Aortic pulse wave velocity**

149 Doppler ultrasound (Indus Mouse Doppler System, Webster, TX) was performed under  
150 isoflurane anesthesia. Mice were placed supine on a heating board with legs secured to

151 electrocardiographic electrodes. Doppler probes (10 and 20 MHz) were placed on the  
152 transverse aortic arch (10 MHz) and abdominal aorta (20 MHz) and the distance between the  
153 probes was determined with a precision caliper. Pre-ejection time, the time between the R-  
154 wave of the electrocardiogram and the foot of the Doppler signal, was determined for each  
155 site. Aortic PWV (aPWV) was calculated by dividing the distance (cm) between the probes  
156 by the difference in pre-ejection times (milliseconds) of the thoracic and abdominal regions.

157

### 158 **Quantification of atherosclerotic lesions**

159 The hearts were prepared as described previously (32) and the atherosclerotic lesions were  
160 estimated according to Paigen et al. (33). Briefly, the hearts were washed in PBS and  
161 incubated in PBS at 4°C for 12 h. Each heart was embedded in OCT compound and stored at -  
162 80°C. One hundred sections of 10 µm thickness were prepared from the top of the left  
163 ventricle, where the aortic valves were first visible, up to a position in the aorta where the  
164 valves were just disappearing from the field. After drying for 2 h, the sections were stained  
165 with Oil Red O (ORO) and counterstained with Mayer's haematoxylin. After ORO staining,  
166 surface lesion areas were measured by computer assisted image quantification using the Leica  
167 QWin software (Leica Microsystems, Wetzlar, Germany). Images were captured with a Sony  
168 (Tokyo, Japan) 3CCD video camera.

169

### 170 **Mouse model of chemically-induced arterial thrombosis**

171 The carotid artery of anesthetized mice was isolated from the surrounding tissue, and placed  
172 forward of a small piece of non-transparent black plastic film to prevent fluorescence  
173 interferences by underlying structures during the visualization of blood flow. Rhodamine 6G  
174 (1 mg/mL) was then retro-orbitally injected. Redox-induced vascular endothelial injury was  
175 achieved by applying a piece of filter paper (2 × 1 mm) saturated with a 7.5% ferric chloride



176 (FeCl<sub>3</sub>) solution directly on the carotid artery for 1 minute. The filter paper was then removed,  
177 and thrombus formation was observed in real time using an epifluorescence microscope  
178 (Olympus BX51WIF, Olympus France S.A.S., Rungis, France) connected to a digital camera  
179 (Olympus CAM-UC90). Time for complete thrombotic occlusion was defined as blood flow  
180 being stopped for at least 30 seconds.

181

## 182 **RNA Extraction and quantitative RT-PCR**

183 For each sample, mouse livers were recovered, flash frozen, and a fraction was grinded in 1  
184 mL of Qiazol (Qiagen, France) with an Ultra Thurax (Imlab, France). Total RNA was  
185 extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, France), and 250 ng were reverse  
186 transcribed using the Verso cDNA Synthesis Kit (ThermoFisher Scientific, France)  
187 according to the manufacturer's instructions. The transcript levels were determined by real-  
188 time quantitative PCR using the CFX 96™ Real-Time System (Biorad, Hercules, CA, USA)  
189 and the SYBR Green Master Mix PCR kit as recommended by the manufacturer  
190 (ThermoFisher Scientific, France). PCR reactions were carried out in duplicates in 96-well  
191 plates (15 µL per well) in a buffer containing 1x SYBR Green mix (including Taq  
192 polymerase, dNTPs, SYBR Green dye), 280 nM forward and reverse primers and 1:10  
193 dilution of reverse transcript RNA. After denaturation at 95°C for 15 min, amplification  
194 occurred in a two-step procedure: 15 s of denaturation at 95°C and 1 min of  
195 annealing/extension at 60°C, with a total of 30 cycles. Identical thermal cycling conditions  
196 were used for all targets. Specific primers were designed using the NCBI Primer Blast  
197 interface and are presented in Table 1. PCR efficiency of the primer sets was calculated by  
198 performing real-time PCR on serial dilutions. For each experiment, PCR reactions were  
199 performed in duplicate and 3 independent experiments were analyzed. Relative gene  
200 expression was determined with the formula fold induction:  $2^{-\Delta\Delta Ct}$ , where  $\Delta\Delta Ct = (Ct_{GI}$

201 [unknown sample]-Ct GI [reference sample]-(Ct reference genes [unknown sample]-Ct  
202 reference genes [reference sample]). GI is the gene of interest. RPS26 and 36B4 are used as  
203 reference genes. The reference samples are ND mice and the unknown samples are HFD  
204 mice.

205

## 206 **Statistical analysis**

207 Data were expressed as mean  $\pm$  standard error of the mean (SEM). *In vitro* and *ex vivo*  
208 parametric data were compared with two-tailed Student *t*-test. *In vivo* non-parametric data  
209 were compared with two-tailed Mann-Whitney *U* test. Multiple comparisons were done by  
210 one-way ANOVA followed by Dunnett's test. P-value  $\leq 0.05$  was considered statistically  
211 significant. Statistical analysis was performed using GraphPad Prism (GraphPad Software, La  
212 Jolla, USA).

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## 225 **Results**

### 226 **LDLR<sup>-/-</sup> mice fed with a HFD for 12 weeks display type 2 diabetes and dyslipidemia**

227 LDLR<sup>-/-</sup> mice were fed with HFD (HFD mice) and compared to ND-fed mice (ND mice).

228 After 12 weeks of diet, HFD mice showed a significant higher body weight than ND mice

229 (31.9±1.1 vs 23.3±0.9) (Fig 1A) and were intolerant to glucose (Fig 1B). In addition, HFD

230 mice showed significant increased blood glucose (8.40±0.78 vs 4.33±0.18 mmol/L), HbA<sub>1c</sub>

231 (25.0±0.4 vs 20.4±1.4 mmol/mol), and insulin (0.128±0.024 vs 0.057±0.015 µg/L)

232 concentrations (Fig 1C, D, E). The HOMA-IR index was also significantly increased in HFD

233 mice (1.02±0.12 vs 0.34±0.09) (Fig 1F). Total cholesterol (24.57±1.50 vs 15.88±0.79

234 mmol/L), HDL- (5.07±0.16 vs 3.22±0.12 mmol/L) and LDL-cholesterol (15.65±0.87 vs

235 10.15±0.53 mmol/L) concentrations were also significantly increased compared to ND mice

236 (Fig 1G, H, I). Unexpectedly, no significant difference was found in free fatty acids and

237 triglycerides plasma levels between HFD and ND mice (Supplemental Digital Content 1).

238 Taken together, these results demonstrated that HFD mice are dyslipidemic and present all the

239 characteristics of type 2 diabetes (hyperglycemia, glucose intolerance, insulin resistance).

240

### 241 **HFD mice display elastic fibers fragmentation and increased aortic pulse wave velocity**

242 We next evaluated the impact of HFD on extracellular matrix remodeling in the aorta of mice

243 by measuring elastic fibers fragmentation. Elastin has autofluorescent properties and emits

244 fluorescence at 500- 560 nm when excited at 488 nm (31) (Fig 2A). In HFD mice, the main

245 number of breaks per elastic lamella was significantly increased compared to ND mice

246 (1.4±0.1 vs 0.3±0.1 breaks, Fig 2B). Moreover, the repartition of the number of breaks per

247 elastic lamella was also strongly different. In ND mice, up to 2 breaks per elastic lamella was

248 observed with the following repartition: no break (72%), 1 break (23%) and 2 breaks (5%)

249 (Fig 2C). In contrast, up to 6 breaks per elastic lamella was observed in HFD mice with the  
250 following repartition: no break (31%), 1 (30%), 2 (18%), 3 (12%), 4 (6%), 5 (2%) and 6  
251 breaks (<1%) (Fig 2C). Taken together, these results demonstrated that HFD induced vascular  
252 extracellular matrix remodeling and fragmentation of elastic fibers in the aorta of mice.  
253 Finally, we monitored the evolution of aPWV in HFD mice (Fig 2D). At baseline, aPWV was  
254 calculated at  $0.365 \pm 0.024$  cm/ms. No major modification was observed between baseline and  
255 9 weeks of HFD ( $0.377 \pm 0.024$  cm/ms). After 12 weeks, aPWV was significantly increased to  
256  $0.449 \pm 0.021$  cm/ms in HFD mice.

257

## 258 **Administration of oseltamivir phosphate in HFD mice has limited effects on type 2** 259 **diabetes and dyslipidemia**

260 The impact of OP administration was next evaluated in LDLR<sup>-/-</sup> mice fed with the same HFD  
261 during 12 weeks. OP (50 mg/kg, + OP) or vehicle (- OP) were administrated weekly by  
262 intraperitoneal injections, starting at the onset of HFD. At this concentration (50 mg/kg), OP  
263 was previously shown to completely ablate tumor vascularization, tumor growth and spread to  
264 the lungs in a mouse model of heterotopic xenografts of MDA-MB-231 tumors (13). As  
265 shown in Fig 3A, B, C, no significant difference was observed on glycemia, insulinemia, and  
266 HOMA-IR index between OP- and vehicle-treated groups, although a trend for increased  
267 insulinemia and HOMA-IR index was noted. Body weights (Fig 3G), HbA<sub>1c</sub>, free fatty acids  
268 and triglycerides concentrations (Supplemental Digital Content 2) were also comparable  
269 between OP- and vehicle-treated mice. When considering plasma cholesterol (Fig 3D, E, F), a  
270 significant decrease of total-cholesterol concentrations was observed in OP-treated group  
271 when compared to vehicle-treated group ( $19.95 \pm 1.29$  vs  $24.57 \pm 1.50$  mmol/L). This was  
272 associated with a significant reduction of LDL-cholesterol concentrations in OP-treated mice  
273 ( $12.98 \pm 0.78$  vs  $15.65 \pm 0.67$  mmol/L).

274

275 **Administration of oseltamivir phosphate in HFD mice decreases elastic fibers**

276 **fragmentation without modification of pulse wave velocity**

277 We next evaluated the effects of OP treatment on elastic fibers fragmentation and aPWV. As  
278 shown in Fig 2B, HFD induces a significant increase in elastic fibers breaks compared to ND.  
279 In HFD mice, OP administration was able to significantly decrease the main number of breaks  
280 per elastic lamella compared to vehicle-treated mice ( $0.9\pm 0.1$  vs  $1.7\pm 0.3$ , Fig 4A). This was  
281 associated with major differences in the repartition of the number of breaks per elastic lamella  
282 (Fig 4B). In vehicle-treated mice, up to 7 breaks per elastic lamella was observed. In OP-  
283 treated mice, no more than 4 breaks were observed. The repartition of elastin breaks was as  
284 follows for the vehicle-treated group: no break (18%), 1 (39%), 2 (21%), 3 (14%), 6 (4%) and  
285 7 breaks (4%). For the OP-treated group, the repartition was no break (40%), 1 (35%), 2  
286 (17%), 3 (6%) and 4 breaks (2%). However, no major impact on the aPWV parameter was  
287 observed between the 2 groups (Fig 4C).

288

289 **Administration of oseltamivir phosphate in HFD mice has no effect on atherosclerosis**  
290 **and carotid thrombosis**

291 After 12 weeks of HFD, LDLR<sup>-/-</sup> mice developed large atherosclerotic lesions in aortic roots  
292 (Fig 5A, left panels). After ORO staining and quantification, the mean lesion size was  
293 estimated at  $0.36\pm 0.03$  mm<sup>2</sup> for vehicle-treated mice and  $0.41\pm 0.02$  mm<sup>2</sup> for OP-treated mice  
294 (Fig 5A, right panels). Together, these data demonstrated that, in our experimental conditions,  
295 OP treatment had no effect on atherosclerosis development. We also evaluated the effects of  
296 OP treatment on arterial thrombosis by using the FeCl<sub>3</sub>-induced carotid artery thrombosis  
297 model (34). Thrombosis was triggered by 7.5% FeCl<sub>3</sub> in both vehicle- and OP-treated HFD  
298 mice and time to complete thrombotic occlusion was monitored using real-time

299 videomicroscopy and defined as an observed arrest in blood flow during at least 30 seconds.  
300 No difference in the mean occlusion time was observed between vehicle- and OP-treated mice  
301 ( $8.5\pm 0.4$  vs  $8.9\pm 0.5$  min) (Fig 5B). Although not significant, a trend for a decrease of the  
302 number of embols released during thrombus formation was observed in the OP-treated group  
303 ( $11.5\pm 3.5$  vs  $5.9\pm 1.4$  embols) (Fig 5C). Taken together, these data demonstrated that, in our  
304 experimental conditions, OP treatment had no effect on carotid thrombosis but may lead to  
305 reduced embolization.

306

### 307 **Administration of oseltamivir phosphate in HFD mice induces liver inflammation and** 308 **fibrosis**

309 After 12 weeks of HFD, the liver weight of LDLR<sup>-/-</sup> mice had more than doubled compared to  
310 mice fed with ND diet ( $1.34\pm 0.07$  vs  $0.61\pm 0.19$  g). No difference was observed between  
311 vehicle- and OP-treated HFD mice (Fig 6A). To evaluate potential toxic effects of OP on the  
312 liver, expression of different inflammation (F4/80, IL-1 $\beta$ , TGF- $\beta$ 1) and extracellular matrix  
313 remodeling (MMP-2, -9, -12, collagen) markers were assessed by quantitative RT-PCR. As  
314 shown in Fig 6B, LDLR<sup>-/-</sup> mice fed with HFD showed a moderate but significant increase by  
315 1.6- and 1.3-fold of IL-1 $\beta$  and TGF- $\beta$ 1 mRNA expression levels, respectively, compared to  
316 ND mice. No significant difference was observed for the macrophage F4/80 marker. In OP-  
317 treated HFD mice, F4/80, IL-1 $\beta$ , TGF- $\beta$ 1 mRNA expression levels were further significantly  
318 increased by 1.7-, 1.7- and 1.8-fold, respectively, compared to vehicle-treated HFD mice (Fig  
319 6B). When considering markers of extracellular matrix remodeling, HFD mice induced a  
320 significant increase by 30-fold of MMP-12 mRNA expression levels compared to ND mice,  
321 and no significant difference was observed for MMP-2, -9 and type I collagen (alpha 1 chain)  
322 (Fig 6C). However, OP treatment further significantly increased by 1.7- and 2.5-fold MMP-12  
323 and collagen mRNA expression levels, respectively, compared to vehicle-treated HFD mice

324 (Fig 6C). Together, these results showed that OP administration to HFD-fed LDLR<sup>-/-</sup> mice  
325 could trigger liver inflammation, extracellular matrix remodeling and fibrosis.

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## 349 **Discussion**

350 Together with zanamivir, OP form the basis of clinically available flu antiviral therapeutics  
351 (35). Whereas viral sialidase inhibitors have also broad specificity for bacterial NEUs, studies  
352 that have assessed the activity of zanamivir and OP against human NEUs (hNEUs) have  
353 reported weaker efficacy (9, 10). In a recent study, IC<sub>50</sub> values for OP were estimated at more  
354 than 500 μM against the four hNEUs, and the only viral NEU inhibitor tested with notable  
355 activity against hNEU2, hNEU3 and hNEU4 was zanamivir (10). However, despite this low  
356 inhibitory potency of OP for hNEUs, several reports have evaluated its use as potential  
357 inhibitor of hNEU1 sialidase activity and related signaling pathways in *in vitro* and *in vivo*  
358 studies. Among the four hNEUs described so far, NEU1 is indeed the most studied sialidase.  
359 Firstly identified in lysosomes (2), NEU1 is also found at the plasma membrane of cells and  
360 regulates a myriad of membrane receptors by desialylation resulting in either activation or  
361 inhibition of the receptors (36-44). At the plasma membrane, NEU1 also associates with the  
362 elastin-binding protein (EBP) and the carboxypeptidase protective protein/cathepsin A  
363 (PPCA) forming the elastin receptor complex (ERC) (45). NEU1 is known to be required for  
364 elastogenesis and signal transduction through this receptor (45-47), and for the biological  
365 effects mediated by the elastin-derived peptides (48, 49).

366 Implication of NEU1 in atherosclerosis is supported by different studies. High expression of  
367 NEU1 has been reported in peripheral blood mononuclear cells of patients with acute  
368 myocardial infarction and in macrophages present in the intima layer, in calcified regions and  
369 within the adventitia of the plaque region in human carotid arteries (24). Enhanced NEU1  
370 expression induces a pro-inflammatory phenotype, triggers production and release of  
371 cytokines and chemokines in monocytes, and promotes phagocytosis and cytokine expression  
372 in macrophages (24). In human macrophages, NEU1 also modulates the sialylation level of  
373 CD36 receptors and enhances oxidized LDL uptake in response to elastin-derived peptides



374 (41). Direct evidence for NEU1 involvement in atherosclerosis came from the works of  
375 Gayral et al (23). Using chimeric LDLR<sup>-/-</sup> mice devoid of NEU1 activity in the  
376 haematopoietic lineage, Gayral et al showed decreased atheroma plaque size progression in  
377 aortic sinus and leucocytes infiltration. Moreover, in ApoE<sup>-/-</sup> mice expressing hypomorphic  
378 levels of NEU1 (*Neu1<sup>hypo</sup>ApoE<sup>-/-</sup>*), White et al have reported reduced serum levels of very-  
379 low-density lipoprotein (VLDL) and LDL-cholesterol, decreased infiltration of inflammatory  
380 cells into lesions, and significant reduction of atherosclerosis (25). Consistent with these  
381 findings, administration of the broad spectrum sialidase inhibitor DANA had anti-atherogenic  
382 effect in the ApoE<sup>-/-</sup> mice (25). Taken together, all these studies suggest that targeting NEU1  
383 expression or catalytic activity may represent a promising target for managing atherosclerosis.  
384 In light of these promising data, we evaluated in this study the effects of OP administration on  
385 atherosclerosis development in HFD-fed LDLR<sup>-/-</sup> mice, at a concentration (50 mg/kg) that  
386 completely ablates tumor vascularization, tumor growth and spread to the lungs in a mouse  
387 model of heterotopic xenografts of MDA-MB-231 tumors (13) and twice lower than the one  
388 used by Gilmour et al in a mouse model of heterotopic xenografts of human pancreatic cancer  
389 (12). From the results presented here, it is suggested that OP treatment has no protective  
390 effect on atherosclerosis development since comparable atheroma plaque size was observed in  
391 aortic sinus of vehicle- and OP-treated LDLR<sup>-/-</sup> mice. If these contradictory results rely on  
392 differences between diet formulation, diet duration and/or mice genotype remain to be further  
393 evaluated. In the present study, LDLR<sup>-/-</sup> mice were fed for 12 weeks with high-fat, high-  
394 carbohydrate diet, and one may consider that the protocol used here was too drastic to see any  
395 potential benefit of OP treatment. Moreover, OP was administrated weekly and not daily as  
396 described previously (13), and one may speculate that the time between two injections has to  
397 be shorten. However, using this protocol, OP was shown to be effective in reducing plasma  
398 LDL-cholesterol levels, in agreement with the works of White et al showing that ApoE<sup>-/-</sup> mice

399 expressing hypomorphic levels of NEU1 had reduced serum levels of LDL-cholesterol (25).  
400 Taken together, these results show that inhibition of NEUs by OP modulates lipoprotein  
401 metabolism and reduces LDL-cholesterol levels. Modification of sialic acids on  
402 apolipoproteins is known to affect lipoprotein metabolism (50-52), and advanced  
403 desialylation of LDL by sialidases is considered as a primary step of atherogenic modification  
404 of lipids (53).

405 Here, we also observed that OP-treated HFD mice are prone to develop insulin resistance.  
406 Even if the difference failed to be significant between vehicle- and OP-treated groups, a near  
407 2-fold increase of the HOMA-IR index was observed for OP-treated mice. Importantly,  
408 insulin receptor activation has been shown to be under the regulation of NEU1 (37, 38) and  
409 mice deficient in NEU1 rapidly develop glucose intolerance and insulin resistance after being  
410 fed with HFD (38). Further works have uncovered a crosstalk between NEU1 and MMP-9 in  
411 alliance with G-protein coupled receptor (GPCR), which was essential for insulin-induced IR  
412 activation and cellular signaling (21, 22).

413 Another interesting finding from this study comes from the protective effect of OP on elastic  
414 fibers integrity in aorta. Elastic fibers are fundamental for mechanical properties of the arterial  
415 wall, and elastic fibers fragmentation has major consequences on atherosclerosis progression  
416 (26). Degradation of elastin involves elastases such as MMPs (MMP-2, -7, -9, -12), cysteine  
417 cathepsins (L, S, K, V), and/or the neutrophil elastase, (54). Whether OP reduces expression  
418 or activity of elastases in aorta remains to be further evaluated. Damaged or degraded elastic  
419 fibers are generally not repaired and replaced by collagens and proteoglycans that stiffen the  
420 arterial wall. Even if no effect was observed on aPWV, OP treatment may help in limiting  
421 arterial stiffness occurring with atherosclerosis. Decreasing elastin fragmentation may also  
422 limit the generation of elastin-degradation products, which are critical players of  
423 atherosclerosis development (26).

424 One major issue of atherosclerosis is plaque rupture and thrombosis. Since desialylation  
425 regulates platelet activation and aggregation (55, 56), we hypothesized that OP treatment may  
426 decrease atherothrombosis. However, we did not observe any difference in our chemically-  
427 induced carotid thrombosis model between vehicle- and OP-treated groups. In contrast, and  
428 even if the difference failed to be significant, a near 2-fold decrease of the number of embols  
429 released during thrombus formation was observed in OP-treated HFD mice. These results,  
430 that remain to be further confirmed, may suggest that OP could reduce embolization and  
431 hence increase thrombus stability.

432 The use of OP in patients has been associated with adverse effects such as nausea, vomiting,  
433 psychiatric effects, renal events (57), and liver toxicity (9, 27-29). Here, we report that OP  
434 treatment has adverse effects on the liver and significantly increased mRNA expression levels  
435 of typical inflammation markers such as the pan-marker of murine macrophages F4/80, and  
436 the pro-inflammatory cytokines IL-1 $\beta$  and TGF- $\beta$ 1. Together with increase in mRNA  
437 expression levels of MMP-12 and type I collagen, our results suggest that OP treatment also  
438 triggers ECM remodeling in the liver and fibrogenic responses. Long-term consequences on  
439 potential steatohepatitis development associated with OP treatment remain to be evaluated.

440

441 In conclusion, the present study shows that, in these experimental settings, OP treatment has  
442 limited effects on atherosclerosis and related thrombotic events in LDLR<sup>-/-</sup> mice. Although  
443 higher concentrations and daily injections have to be considered before stating that OP has no  
444 protective effect on atherosclerosis and thrombosis, these data suggest that caution must be  
445 taken concerning its side effects on liver. Even if promising effects of OP have been reported  
446 in different animal models of human diseases, benefits versus harms of OP remains to be  
447 further evaluated before potential application in human.

448

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452

453 **Conflict of interest**

454 The authors declare that they have no conflict interest.

455

456 **Author contributions**

457 PM conceived, designed, and supervised the study. LD, SB and ML helped in designing the  
458 study. OB, AW, TS, EV, CS, AF, SG, AH, SB, BRC and PM performed the experiments. AB,  
459 HS, SJ, PG and LM contributed to discussion. PM and LD were involved in writing the  
460 manuscript and PM and CS prepared the figures.

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## 473 **References**

- 474 1. Miyagi T, Yamaguchi K. Mammalian sialidases: physiological and pathological roles  
475 in cellular functions. *Glycobiology*. 2012;22(7):880-96.
- 476 2. Bonten E, van der Spoel A, Fornerod M, Grosveld G, d'Azzo A. Characterization of  
477 human lysosomal neuraminidase defines the molecular basis of the metabolic storage disorder  
478 sialidosis. *Genes Dev*. 1996;10(24):3156-69.
- 479 3. Monti E, Preti A, Rossi E, Ballabio A, Borsani G. Cloning and characterization of  
480 NEU2, a human gene homologous to rodent soluble sialidases. *Genomics*. 1999;57(1):137-43.
- 481 4. Wada T, Yoshikawa Y, Tokuyama S, Kuwabara M, Akita H, Miyagi T. Cloning,  
482 expression, and chromosomal mapping of a human ganglioside sialidase. *Biochem Biophys*  
483 *Res Commun*. 1999;261(1):21-7.
- 484 5. Monti E, Bassi MT, Bresciani R, Civini S, Croci GL, Papini N, et al. Molecular  
485 cloning and characterization of NEU4, the fourth member of the human sialidase gene family.  
486 *Genomics*. 2004;83(3):445-53.
- 487 6. Glanz VY, Myasoedova VA, Grechko AV, Orekhov AN. Sialidase activity in human  
488 pathologies. *Eur J Pharmacol*. 2019;842:345-50.
- 489 7. Bassetti M, Castaldo N, Carnelutti A. Neuraminidase inhibitors as a strategy for  
490 influenza treatment: pros, cons and future perspectives. *Expert Opin Pharmacother*.  
491 2019;20(14):1711-8.
- 492 8. Wilson JC, von Itzstein M. Recent strategies in the search for new anti-influenza  
493 therapies. *Curr Drug Targets*. 2003;4(5):389-408.
- 494 9. Hata K, Koseki K, Yamaguchi K, Moriya S, Suzuki Y, Yingsakmongkon S, et al.  
495 Limited inhibitory effects of oseltamivir and zanamivir on human sialidases. *Antimicrob*  
496 *Agents Chemother*. 2008;52(10):3484-91.

- 497 10. Richards MR, Guo T, Hunter CD, Cairo CW. Molecular dynamics simulations of viral  
498 neuraminidase inhibitors with the human neuraminidase enzymes: Insights into isoenzyme  
499 selectivity. *Bioorg Med Chem*. 2018;26(19):5349-58.
- 500 11. Abdulkhalek S, Geen OD, Brodhagen L, Haxho F, Alghamdi F, Allison S, et al.  
501 Transcriptional factor snail controls tumor neovascularization, growth and metastasis in  
502 mouse model of human ovarian carcinoma. *Clin Transl Med*. 2014;3(1):28.
- 503 12. Gilmour AM, Abdulkhalek S, Cheng TS, Alghamdi F, Jayanth P, O'Shea LK, et al. A  
504 novel epidermal growth factor receptor-signaling platform and its targeted translation in  
505 pancreatic cancer. *Cell Signal*. 2013;25(12):2587-603.
- 506 13. Haxho F, Allison S, Alghamdi F, Brodhagen L, Kuta VE, Abdulkhalek S, et al.  
507 Oseltamivir phosphate monotherapy ablates tumor neovascularization, growth, and metastasis  
508 in mouse model of human triple-negative breast adenocarcinoma. *Breast Cancer (Dove Med  
509 Press)*. 2014;6:191-203.
- 510 14. Haxho F, Neufeld RJ, Szewczuk MR. Neuraminidase-1: a novel therapeutic target in  
511 multistage tumorigenesis. *Oncotarget*. 2016;7(26):40860-81.
- 512 15. Wood K, Szewczuk MR, Rousseau D, Neufeld RJ. Oseltamivir phosphate released  
513 from injectable Pickering emulsions over an extended term disables human pancreatic cancer  
514 cell survival. *Oncotarget*. 2018;9(16):12754-68.
- 515 16. O'Shea LK, Abdulkhalek S, Allison S, Neufeld RJ, Szewczuk MR. Therapeutic  
516 targeting of Neu1 sialidase with oseltamivir phosphate (Tamiflu(R)) disables cancer cell  
517 survival in human pancreatic cancer with acquired chemoresistance. *Onco Targets Ther*.  
518 2014;7:117-34.
- 519 17. Thulasiraman P, Kerr K, McAlister K, Hardisty S, Wistner A, McCullough I.  
520 Neuraminidase 1 regulates proliferation, apoptosis and the expression of Cadherins in  
521 mammary carcinoma cells. *Mol Cell Biochem*. 2019;462(1-2):207-15.

- 522 18. Abdulkhalek S, Szewczuk MR. Neu1 sialidase and matrix metalloproteinase-9 cross-  
523 talk regulates nucleic acid-induced endosomal TOLL-like receptor-7 and -9 activation,  
524 cellular signaling and pro-inflammatory responses. *Cell Signal*. 2013;25(11):2093-105.
- 525 19. Amith SR, Jayanth P, Franchuk S, Siddiqui S, Seyrantepe V, Gee K, et al. Dependence  
526 of pathogen molecule-induced toll-like receptor activation and cell function on Neu1  
527 sialidase. *Glycoconj J*. 2009;26(9):1197-212.
- 528 20. Finlay TM, Abdulkhalek S, Gilmour A, Guzzo C, Jayanth P, Amith SR, et al.  
529 Thymoquinone-induced Neu4 sialidase activates NFkappaB in macrophage cells and pro-  
530 inflammatory cytokines in vivo. *Glycoconj J*. 2010;27(6):583-600.
- 531 21. Alghamdi F, Guo M, Abdulkhalek S, Crawford N, Amith SR, Szewczuk MR. A novel  
532 insulin receptor-signaling platform and its link to insulin resistance and type 2 diabetes. *Cell*  
533 *Signal*. 2014;26(6):1355-68.
- 534 22. Haxho F, Haq S, Szewczuk MR. Biased G protein-coupled receptor agonism mediates  
535 Neu1 sialidase and matrix metalloproteinase-9 crosstalk to induce transactivation of insulin  
536 receptor signaling. *Cell Signal*. 2018;43:71-84.
- 537 23. Gayral S, Garnotel R, Castaing-Berthou A, Blaise S, Fougerat A, Berge E, et al.  
538 Elastin-derived peptides potentiate atherosclerosis through the immune Neu1-PI3Kgamma  
539 pathway. *Cardiovasc Res*. 2014;102(1):118-27.
- 540 24. Sieve I, Ricke-Hoch M, Kasten M, Battmer K, Stapel B, Falk CS, et al. A positive  
541 feedback loop between IL-1beta, LPS and NEU1 may promote atherosclerosis by enhancing a  
542 pro-inflammatory state in monocytes and macrophages. *Vascul Pharmacol*. 2018;103-105:16-  
543 28.
- 544 25. White EJ, Gyulay G, Lhotak S, Szewczyk MM, Chong T, Fuller MT, et al. Sialidase  
545 down-regulation reduces non-HDL cholesterol, inhibits leukocyte transmigration, and  
546 attenuates atherosclerosis in ApoE knockout mice. *J Biol Chem*. 2018;293(38):14689-706.

- 547 26. Duca L, Blaise S, Romier B, Laffargue M, Gayral S, El Btaouri H, et al. Matrix ageing  
548 and vascular impacts: focus on elastin fragmentation. *Cardiovasc Res.* 2016;110(3):298-308.
- 549 27. El-Sayed WM, Al-Kahtani MA. Potential adverse effects of oseltamivir in rats: males  
550 are more vulnerable than females. *Can J Physiol Pharmacol.* 2011;89(9):623-30.
- 551 28. Fang S, Qi L, Zhou N, Li C. Case report on alimentary tract hemorrhage and liver  
552 injury after therapy with oseltamivir: A case report. *Medicine (Baltimore).*  
553 2018;97(38):e12497.
- 554 29. Hama R. The mechanisms of delayed onset type adverse reactions to oseltamivir.  
555 *Infect Dis (Lond).* 2016;48(9):651-60.
- 556 30. Heikkinen S, Argmann CA, Champy MF, Auwerx J. Evaluation of glucose  
557 homeostasis. *Curr Protoc Mol Biol.* 2007;Chapter 29:Unit 29B 3.
- 558 31. Monici M. Cell and tissue autofluorescence research and diagnostic applications.  
559 *Biotechnol Annu Rev.* 2005;11:227-56.
- 560 32. Fougerat A, Pan X, Smutova V, Heveker N, Cairo CW, Issad T, et al. Neuraminidase  
561 1 activates insulin receptor and reverses insulin resistance in obese mice. *Mol Metab.*  
562 2018;12:76-88.
- 563 33. Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA. Quantitative assessment  
564 of atherosclerotic lesions in mice. *Atherosclerosis.* 1987;68(3):231-40.
- 565 34. Li W, McIntyre TM, Silverstein RL. Ferric chloride-induced murine carotid arterial  
566 injury: A model of redox pathology. *Redox Biol.* 2013;1:50-5.
- 567 35. von Itzstein M. The war against influenza: discovery and development of sialidase  
568 inhibitors. *Nat Rev Drug Discov.* 2007;6(12):967-74.
- 569 36. Amith SR, Jayanth P, Franchuk S, Finlay T, Seyrantepe V, Beyaert R, et al. Neu1  
570 desialylation of sialyl alpha-2,3-linked beta-galactosyl residues of TOLL-like receptor 4 is  
571 essential for receptor activation and cellular signaling. *Cell Signal.* 2010;22(2):314-24.



572 37. Blaise S, Romier B, Kawecki C, Ghirardi M, Rabenoelina F, Baud S, et al. Elastin-  
573 derived peptides are new regulators of insulin resistance development in mice. *Diabetes*.  
574 2013;62(11):3807-16.

575 38. Dridi L, Seyrantepe V, Fougerat A, Pan X, Bonneil E, Thibault P, et al. Positive  
576 regulation of insulin signaling by neuraminidase 1. *Diabetes*. 2013;62(7):2338-46.

577 39. Hinek A, Bodnaruk TD, Bunda S, Wang Y, Liu K. Neuraminidase-1, a subunit of the  
578 cell surface elastin receptor, desialylates and functionally inactivates adjacent receptors  
579 interacting with the mitogenic growth factors PDGF-BB and IGF-2. *Am J Pathol*.  
580 2008;173(4):1042-56.

581 40. Jayanth P, Amith SR, Gee K, Szewczuk MR. Neu1 sialidase and matrix  
582 metalloproteinase-9 cross-talk is essential for neurotrophin activation of Trk receptors and  
583 cellular signaling. *Cell Signal*. 2010;22(8):1193-205.

584 41. Kawecki C, Bocquet O, Schmelzer CEH, Heinz A, Ihling C, Wahart A, et al.  
585 Identification of CD36 as a new interaction partner of membrane NEU1: potential implication  
586 in the pro-atherogenic effects of the elastin receptor complex. *Cell Mol Life Sci*.  
587 2019;76(4):791-807.

588 42. Lee C, Liu A, Miranda-Ribera A, Hyun SW, Lillehoj EP, Cross AS, et al. NEU1  
589 sialidase regulates the sialylation state of CD31 and disrupts CD31-driven capillary-like tube  
590 formation in human lung microvascular endothelia. *J Biol Chem*. 2014;289(13):9121-35.

591 43. Lillehoj EP, Hyun SW, Feng C, Zhang L, Liu A, Guang W, et al. NEU1 sialidase  
592 expressed in human airway epithelia regulates epidermal growth factor receptor (EGFR) and  
593 MUC1 protein signaling. *J Biol Chem*. 2012;287(11):8214-31.

594 44. Uemura T, Shiozaki K, Yamaguchi K, Miyazaki S, Satomi S, Kato K, et al.  
595 Contribution of sialidase NEU1 to suppression of metastasis of human colon cancer cells  
596 through desialylation of integrin beta4. *Oncogene*. 2009;28(9):1218-29.

- 597 45. Hinek A, Pshezhetsky AV, von Itzstein M, Starcher B. Lysosomal sialidase  
598 (neuraminidase-1) is targeted to the cell surface in a multiprotein complex that facilitates  
599 elastic fiber assembly. *J Biol Chem.* 2006;281(6):3698-710.
- 600 46. Duca L, Blanchevoye C, Cantarelli B, Ghoneim C, Dedieu S, Delacoux F, et al. The  
601 elastin receptor complex transduces signals through the catalytic activity of its Neu-1 subunit.  
602 *J Biol Chem.* 2007;282(17):12484-91.
- 603 47. Rusciani A, Duca L, Sartelet H, Chatron-Colliet A, Bobichon H, Ploton D, et al.  
604 Elastin peptides signaling relies on neuraminidase-1-dependent lactosylceramide generation.  
605 *PLoS One.* 2010;5(11):e14010.
- 606 48. Bennisroune A, Romier-Crouzet B, Blaise S, Laffargue M, Efremov RG, Martiny L,  
607 et al. Elastic fibers and elastin receptor complex: Neuraminidase-1 takes the center stage.  
608 *Matrix Biol.* 2019;84:57-67.
- 609 49. Wahart A, Hocine T, Albrecht C, Henry A, Sarazin T, Martiny L, et al. Role of elastin  
610 peptides and elastin receptor complex in metabolic and cardiovascular diseases. *FEBS J.*  
611 2019;286(15):2980-93.
- 612 50. Filipovic I, Schwarzmann G, Mraz W, Wiegandt H, Buddecke E. Sialic-acid content  
613 of low-density lipoproteins controls their binding and uptake by cultured cells. *Eur J*  
614 *Biochem.* 1979;93(1):51-5.
- 615 51. Millar JS. The sialylation of plasma lipoproteins. *Atherosclerosis.* 2001;154(1):1-13.
- 616 52. Orekhov AN, Tertov VV, Sobenin IA, Smirnov VN, Via DP, Guevara J, Jr., et al.  
617 Sialic acid content of human low density lipoproteins affects their interaction with cell  
618 receptors and intracellular lipid accumulation. *J Lipid Res.* 1992;33(6):805-17.
- 619 53. Zhang C, Chen J, Liu Y, Xu D. Sialic acid metabolism as a potential therapeutic target  
620 of atherosclerosis. *Lipids Health Dis.* 2019;18(1):173.

621 54. Maurice P, Blaise S, Gayral S, Debelle L, Laffargue M, Hornebeck W, et al. Elastin  
622 fragmentation and atherosclerosis progression: the elastokine concept. *Trends Cardiovasc*  
623 *Med.* 2013;23(6):211-21.

624 55. Kullaya V, de Jonge MI, Langereis JD, van der Gaast-de Jongh CE, Bull C, Adema  
625 GJ, et al. Desialylation of Platelets by Pneumococcal Neuraminidase A Induces ADP-  
626 Dependent Platelet Hyperreactivity. *Infect Immun.* 2018;86(10).

627 56. van der Wal DE, Davis AM, Mach M, Marks DC. The role of neuraminidase 1 and 2  
628 in glycoprotein Ibalpha-mediated integrin alphaIIbbeta3 activation. *Haematologica.*  
629 2020;105(4):1081-94.

630 57. Jefferson T, Jones MA, Doshi P, Del Mar CB, Hama R, Thompson MJ, et al.  
631 Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children.  
632 *Cochrane Database Syst Rev.* 2014(4):CD008965.

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646 **Figure legends**

647 **Figure 1. Impact of HFD on body weight gain and metabolic parameters in LDLR<sup>-/-</sup>**  
648 **mice.**

649 Body weight (A), glucose tolerance test (B), plasma glucose (C), HbA<sub>1C</sub> (D) plasma insulin  
650 (E), HOMA-IR index (F), and plasma total- (G), HDL- (H), and LDL- (I) cholesterol  
651 concentrations were measured from fasted LDLR<sup>-/-</sup> mice. White bars (A, C-I) and white  
652 circles (B) correspond to mice fed with normal diet (ND), and black bars (A, C-I) and black  
653 squares (B) correspond to mice fed with HFD. Data represent the mean ± SEM of 4 to 5 mice.  
654 (\*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001).

655

656 **Figure 2. Impact of HFD on elastin fragmentation in aorta and aortic pulse wave**  
657 **velocity in LDLR<sup>-/-</sup> mice.**

658 (A) Autofluorescence of aortic elastic fibers in normal diet (ND) and high fat diet (HFD)  
659 mice. White arrows indicate zone of elastin breaks. (B) Quantification of the number of  
660 breaks per elastic lamella in ND (white bar) and HFD (black bar) mice. Data represent the  
661 mean ± SEM of 4 to 5 mice per group (\*\*\*, p<0.001). (C) Percent repartition of the number  
662 of breaks per elastic lamella in ND and HFD mice. The total circle represents 100%. (D)  
663 Evolution of aPWV every 3 months in HFD mice. Data represent the mean ± SEM of 7 to 9  
664 mice. (\*, p<0.05 compared to baseline).

665

666 **Figure 3. Effect of oseltamivir phosphate treatment on body weight and metabolic**  
667 **parameters in HFD-fed LDLR<sup>-/-</sup> mice.**

668 Plasma glucose (A), insulin (B), HOMA-IR index (C), and plasma total- (D), HDL- (E), and  
669 LDL- (F) cholesterol concentrations were measured from fasted LDLR<sup>-/-</sup> mice fed with high fat  
670 diet (HFD) for 12 weeks, and treated (+OP) or not (-OP) with oseltamivir phosphate (50

671 mg/kg, weekly). (G) Body weight. White bars (A-F) and white circles (G) correspond to  
672 vehicle-treated mice, and black bars (A-F) and black squares (G) correspond to OP-treated  
673 mice. Data represent the mean  $\pm$  SEM of 4 to 5 mice. (ns, non-significant; \*,  $p < 0.05$ ).

674

675 **Figure 4. Effect of oseltamivir phosphate treatment on elastin fragmentation in aorta**  
676 **and aortic pulse wave velocity in HFD-fed LDLR<sup>-/-</sup> mice.**

677 (A) Quantification of the number of breaks per elastic lamella in mice treated with vehicle (-  
678 OP, white bar) or OP (+OP, black bar). Data represent the mean  $\pm$  SEM of 4 to 5 mice per  
679 group (\*\*,  $p < 0.01$ ). (B) Percent repartition of the number of breaks per elastic lamella in mice  
680 treated with vehicle (-OP) or OP (+OP). The total circle represents 100%. (C) Evolution of  
681 aPWV every 3 months in HFD mice treated with vehicle (-OP, white circles) or OP (+OP,  
682 black squares). Data represent the mean  $\pm$  SEM of 7 to 10 mice per group.

683

684 **Figure 5. Effect of oseltamivir phosphate treatment on atherosclerosis in aortic sinus**  
685 **and carotid thrombosis in HFD-fed LDLR<sup>-/-</sup> mice.**

686 (A) Quantification of atherosclerotic lesion sizes in the aortic roots of mice treated with  
687 vehicle (-OP) or OP (+OP). Left panel shows a representative photomicrograph of ORO-  
688 stained fatty streaks for each group (original magnification  $\times 50$ ). Right panel shows  
689 quantitative analysis of atherosclerotic lesion sizes ( $\text{mm}^2$ ) for mice treated with vehicle (-OP,  
690 white circles) and OP (+OP, black squares). The mean  $\pm$  SEM is shown. (B) Thrombotic  
691 occlusion time of mice treated with vehicle (-OP, white circles) or OP (+OP, black squares)  
692 after chemical injury of carotid arteries by 7.5 % FeCl<sub>3</sub>. The mean  $\pm$  SEM is shown. Top  
693 photographs show, as an illustration, a representative progression of thrombus formation in  
694 mice injected with vehicle (-OP) or OP (+OP). (C) Number of embols released during  
695 thrombus formation in the carotid arteries of mice treated with vehicle (-OP, white bar) and

696 OP (+OP, black bar). Data represent the mean  $\pm$  SEM of 6 to 7 carotids per group. (ns, non-  
697 significant).

698

699 **Figure 6. RT-qPCR analysis of gene expression in the liver of ND- and HFD-fed LDLR<sup>-/-</sup>**  
700 **mice and impact of oseltamivir phosphate treatment.**

701 (A) Liver weight of mice fed with normal diet (ND, white bar) or high fat diet (HFD) treated  
702 with vehicle (-OP, black bar) or OP (+OP, hatched black bar). Data represent the mean  $\pm$   
703 SEM of 4 to 5 mice per group. (B, C) Total RNA was extracted from mice livers and analyzed  
704 by RT-qPCR as described in the Materials and Methods. Each gene expression was  
705 normalized using 2 reference genes (36B4 and RPS26) and results are presented as fold  
706 induction of ND mice. White bars, ND mice; black bars, HFD mice treated with vehicle;  
707 hatched black bars, HFD mice treated with OP. Data are expressed as mean  $\pm$  SEM of 4 to 5  
708 mice per group. For each mouse, PCR reactions were performed in duplicate from 3  
709 independent experiments. (ns, not significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ).

710