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## **Interaction between Hormone-Sensitive Lipase and ChREBP in Fat Cells Controls Insulin Sensitivity**

Pauline Morigny, Marianne Houssier, Aline Mairal, Claire Ghilain, Etienne Mouisel, Fadila Benhamed, Bernard Masri, Emeline Recazens, Pierre-Damien Denechaud, Geneviève Tavernier, et al.

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1 Interaction between Hormone-Sensitive Lipase and ChREBP in Fat Cells  
2 Controls Insulin Sensitivity

3

4 Pauline Morigny<sup>1,2,26</sup>, Marianne Houssier<sup>1,2,26</sup>, Aline Mairal<sup>1,2</sup>, Claire Ghilain<sup>1,2</sup>, Etienne  
5 Mouisel<sup>1,2</sup>, Fadila Benhamed<sup>3,4,5</sup>, Bernard Masri<sup>1,2</sup>, Emeline Recazens<sup>1,2</sup>, Pierre-Damien  
6 Denechaud<sup>1,2</sup>, Geneviève Tavernier<sup>1,2</sup>, Sylvie Caspar-Bauguil<sup>1,2,6</sup>, Sam Virtue<sup>7</sup>, Veronika  
7 Sramkova<sup>1,2,8,9</sup>, Laurent Monbrun<sup>1,2</sup>, Anne Mazars<sup>1,2</sup>, Madjid Zanoun<sup>1,2</sup>, Sandra Guilmeau<sup>3,4,5</sup>,  
8 Valentin Barquissau<sup>1,2</sup>, Diane Beuzelin<sup>1,2</sup>, Sophie Bonnel<sup>1,2,9</sup>, Marie Marques<sup>1,2,9</sup>, Boris  
9 Monge-Roffarello<sup>1,2</sup>, Corinne Lefort<sup>1,2</sup>, Barbara Fielding<sup>10</sup>, Thierry Sulpice<sup>11</sup>, Arne Astrup<sup>12</sup>,  
10 Bernard Payraastre<sup>1,2</sup>, Justine Bertrand-Michel<sup>1,2</sup>, Emmanuelle Meugnier<sup>13</sup>, Laetitia Ligat<sup>14</sup>,  
11 Frédéric Lopez<sup>14</sup>, Hervé Guillou<sup>15,16</sup>, Charlotte Ling<sup>17</sup>, Cecilia Holm<sup>18</sup>, Remi Rabasa-  
12 Lhoret<sup>19,20,21</sup>, Wim H. M. Saris<sup>22</sup>, Vladimir Stich<sup>8,9</sup>, Peter Arner<sup>23</sup>, Mikael Rydén<sup>23</sup>, Cedric  
13 Moro<sup>1,2,9</sup>, Nathalie Viguerie<sup>1,2,9</sup>, Matthew Harms<sup>24</sup>, Stefan Hallén<sup>24</sup>, Antonio Vidal-Puig<sup>7,25</sup>,  
14 Hubert Vidal<sup>13</sup>, Catherine Postic<sup>3,4,5</sup>, Dominique Langin<sup>1,2,6,9\*</sup>

15

16 <sup>1</sup> Institut National de la Santé et de la Recherche Médicale (Inserm), UMR1048, Institute of  
17 Metabolic and Cardiovascular Diseases, Toulouse, France;

18 <sup>2</sup> University of Toulouse, UMR1048, Institute of Metabolic and Cardiovascular Diseases,  
19 Paul Sabatier University, Toulouse, France;

20 <sup>3</sup> Institut National de la Santé et de la Recherche Médicale (Inserm), U1016, Institut Cochin,  
21 Paris, France;

22 <sup>4</sup> Centre National de la Recherche Scientifique (CNRS), UMR 8104, Paris, France;

23 <sup>5</sup> Université Paris Descartes, Sorbonne Paris Cité, Paris, France;

24 <sup>6</sup> Toulouse University Hospitals, Laboratory of Clinical Biochemistry, Toulouse, France;

25 <sup>7</sup> University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC Institute  
26 of Metabolic Science, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK;

27 <sup>8</sup> Department for the Study of Obesity and Diabetes, Third Faculty of Medicine, Charles  
28 University, Prague, Czech Republic;

29 <sup>9</sup> Franco-Czech Laboratory for Clinical Research on Obesity, Third Faculty of Medicine,  
30 Prague and Paul Sabatier University, Toulouse, France;

31 <sup>10</sup> Department of Nutritional Sciences, University of Surrey, Guildford, Surrey;

32 <sup>11</sup> Physiogenex SAS, Prologue Biotech, Labège, France;

33 <sup>12</sup> Department of Nutrition, Exercise and Sports, Faculty of Science, University of  
34 Copenhagen, Copenhagen, Denmark;

35 <sup>13</sup> CarMeN Laboratory, Inserm U1060, INRA U1397, Université Lyon 1, INSA Lyon,  
36 Oullins, France;

37 <sup>14</sup> Pôle Technologique, Cancer Research Center of Toulouse (CRCT), Plateau Interactions  
38 Moléculaires, INSERM-UMR1037, 31037 Toulouse, France.

39 <sup>15</sup> Institut National de la Recherche Agronomique (INRA), UMR1331, Integrative Toxicology  
40 and Metabolism, Toulouse, France

41 <sup>16</sup> University of Toulouse, UMR1331, Institut National Polytechnique (INP), Paul Sabatier  
42 University, Toulouse, France;

43 <sup>17</sup> Department of Clinical Sciences, Epigenetics and Diabetes, Lund University Diabetes  
44 Centre, Clinical Research Centre, Malmö, Sweden;

45 <sup>18</sup> Department of Experimental Medical Science, Lund University, Biomedical Centre, Lund,  
46 Sweden;

47 <sup>19</sup> Institut de Recherches Cliniques de Montréal, Montreal, Canada;

48 <sup>20</sup> Department of nutrition, Université de Montréal, Montreal, Canada;

49 <sup>21</sup> Montreal Diabetes Research Center (MDRC), Montreal, Canada;

50 <sup>22</sup> Department of Human Biology, NUTRIM School of Nutrition and Translational Research  
51 in Metabolism, Maastricht University Medical Centre, Maastricht, Netherlands;

52 <sup>23</sup> Department of Medicine, H7 Karolinska Institutet and Karolinska University Hospital,  
53 Huddinge, Stockholm, Sweden;

54 <sup>24</sup> Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg,  
55 Sweden;

56 <sup>25</sup> Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton,  
57 Cambridgeshire CB10 1SA, UK;

58 <sup>26</sup> These authors contributed equally to the work.

59

60 \*e-mail : dominique.langin@inserm.fr

61

62 ABSTRACT

63 Impaired adipose tissue insulin signaling is a critical feature of insulin resistance. Here we  
64 identify a pathway linking the lipolytic enzyme, hormone-sensitive lipase (HSL), to insulin  
65 action via the glucose-responsive transcription factor ChREBP and its target, the fatty acid  
66 (FA) elongase, ELOVL6. Genetic inhibition of HSL in human adipocytes and mouse adipose  
67 tissue results in enhanced insulin sensitivity and induction of ELOVL6. ELOVL6 promotes an  
68 increase in phospholipid (PL) oleic acid which modifies plasma membrane fluidity and  
69 enhances insulin signaling. HSL deficiency-mediated effects are suppressed by gene silencing  
70 of ChREBP and ELOVL6. Mechanistically, physical interaction between HSL and  
71 ChREBP $\alpha$ , independently of lipase catalytic activity, impairs ChREBP $\alpha$  translocation into the  
72 nucleus and induction of ChREBP $\beta$ , the transcriptionally highly active isoform strongly  
73 associated to whole body insulin sensitivity. Targeting the HSL-ChREBP interaction may  
74 allow therapeutic strategies for the restoration of insulin sensitivity.

75

76 **Introduction**

77 Insulin resistance is a pathogenic mechanism involved in a wide array of diseases. Besides the  
78 well-established early defect seen in type 2 diabetes, insulin resistance plays a role in the  
79 development of cancers such as colorectal cancer, liver diseases associated with non-alcoholic  
80 steatohepatitis, cardiovascular diseases or, reproductive dysfunction e.g., in polycystic ovary  
81 syndrome. Insulin resistance is also a feature of aging-related disorders such as  
82 neurodegenerative diseases e.g., in Alzheimer disease. Adipose tissue metabolism has  
83 emerged as a major determinant of systemic insulin sensitivity. Genetic ablation of insulin-  
84 induced glucose transport in fat causes systemic insulin resistance <sup>1</sup>. Direct manipulation of  
85 the fat cell insulin signaling pathway in mice also supports the systemic importance of  
86 adipose tissue <sup>2-4</sup>. Defects in adipose insulin signaling have been reported in insulin resistant  
87 and type 2 diabetic patients <sup>5-8</sup>. In this context, improvement of adipose tissue insulin action  
88 appears to be an important target for recovery of whole body systemic insulin sensitivity.  
89 Glitazones, a well-known class of insulin sensitizers, act through modulation of fat cell insulin  
90 sensitivity <sup>9,10</sup>. Given their side-effects and subsequent withdrawal in many countries, there is  
91 an unmet need of drugs targeting adipose tissue. Excessive circulating levels of fatty acids are  
92 considered as important contributors to insulin resistance through development of fatty acid-  
93 induced lipotoxicity in insulin-sensitive tissues such as liver and skeletal muscle <sup>11</sup>. Lowering  
94 of plasma non-esterified fatty acid levels through inhibition of fat cell lipolysis has been  
95 proposed as an approach to improve insulin sensitivity. However, human data questions the  
96 association between production of fatty acids from adipose tissue lipolysis and insulin  
97 resistance in obesity <sup>12</sup>. Partial deficiency in HSL (encoded by *LIPE*), one of the neutral  
98 lipases expressed in adipocytes, results in improvement of whole body insulin sensitivity in  
99 obese mice without changes in plasma fatty acid levels suggesting that other mechanisms than  
100 lipolysis are involved <sup>13</sup>.

101 Here, in a series of in vitro and in vivo studies in humans and mice, we identified a pathway  
102 linking HSL to insulin resistance through interaction with the glucose-responsive transcription  
103 factor ChREBP. The physical interaction between HSL and ChREBP impairs nuclear  
104 translocation and activity of the transcription factor. In fat cells, the lipogenic enzyme  
105 ELOVL6 is a preferential target of ChREBP. Inhibition of HSL promotes activity of ELOVL6  
106 and enhances insulin signaling through enrichment of plasma membrane phospholipids in  
107 oleic acid.

108

### 109 **Reduction in HSL expression promotes *de novo* lipogenesis and insulin signaling in** 110 **human adipocytes**

111 In adipocytes differentiated from human multipotent adipose-derived stem (hMADS) cells  
112 <sup>14,15</sup>, HSL gene silencing (**Supplementary Fig. 1a,b,c**) increased insulin-stimulated glucose  
113 transport (**Fig. 1a**), glucose oxidation (**Fig. 1b**) and glucose carbon incorporation into FA, i.e.  
114 *de novo* lipogenesis (**Fig. 1c**). Insulin signaling was enhanced in adipocytes with decreased  
115 HSL expression as shown by enhanced activating phosphorylations of insulin receptor  
116 substrate 1 (IRS1-pY612) and V-Akt murine thymoma viral oncogene homolog  
117 (AKT)/protein kinase B (AKT-pS473, AKT-pT308) after insulin treatment (**Fig. 1d,e,f**).  
118 Phosphorylation of AS160, an AKT substrate regulating translocation of the insulin-sensitive  
119 glucose transporter GLUT4, showed a trend similar to IRS1 and AKT phosphorylation (**Fig.**  
120 **1g**). As adipose tissue *de novo* lipogenesis is associated with insulin sensitivity in humans  
121 <sup>16,17</sup>, we tested whether direct inhibition of *de novo* lipogenesis has an impact on the  
122 modulation of insulin signaling induced by HSL depletion. To this end, human adipocytes  
123 were treated with a selective inhibitor of FA synthase, the rate-limiting enzyme in the  
124 synthesis of palmitic acid (**Supplementary Fig. 1d**). The FA synthase inhibitor blunted the  
125 induction of insulin-mediated phosphorylation of AKT observed in HSL-deficient fat cells

126 **(Supplementary Fig. 1e)**. To further probe the role of *de novo* lipogenesis, we analyzed FA  
127 composition in fat cell triglycerides (TG) and PL. HSL inhibition significantly decreased the  
128 proportion of palmitic acid and palmitoleic acid but increased that of oleic acid in TG and PL  
129 **(Fig. 1h,i)**.

130 To define the molecular mechanisms underlying the changes in FA composition, we analyzed  
131 gene expression of enzymes catalyzing key steps in the synthesis of the main saturated and  
132 monounsaturated FA derived from glucose in human fat cells **(Supplementary Fig. 1d)**. In  
133 hMADS adipocytes with decreased HSL expression, the most robust induction was observed  
134 for *ELOVL6* **(Fig. 1j)**. The increase in *ELOVL6* mRNA level was mirrored by an increase in  
135 enzyme activity **(Supplementary Fig. 1f)** and an increase in the FA elongation ratio  
136 attributable to *ELOVL6* activity **(Supplementary Fig. 1g)**. To confirm data from hMADS  
137 adipocytes, we performed HSL gene silencing in human preadipocytes differentiated in  
138 primary cultures. *ELOVL6* also showed the highest induction among *de novo* lipogenesis  
139 genes **(Supplementary Fig. 1h)**. Next, we evaluated the effect of adipose triglyceride lipase  
140 (ATGL encoded by *PNPLA2*), through ATGL gene silencing in hMADS adipocytes  
141 **(Supplementary Fig. 1i,j)**. ATGL precedes HSL in the sequential breakdown of TG during  
142 adipocyte lipolysis. Contrarily to what is observed during HSL depletion, ATGL knock down  
143 had no effect on *ELOVL6* and other *de novo* lipogenic enzyme mRNA levels as well as on the  
144 FA elongation ratio attributable to *ELOVL6* activity **(Supplementary Fig. 1k,l)**. Altogether,  
145 the results show that HSL depletion improves insulin signaling and, promotes *de novo*  
146 lipogenesis and modification in FA composition. These changes are associated with induction  
147 of the FA elongase *ELOVL6*.

148

149 **HSL inhibition is associated with improved insulin sensitivity and increased adipose**  
150 **tissue *Elovl6* expression in vivo**



151 To probe changes in insulin sensitivity upon decreased HSL expression in vivo, we  
152 investigated different mouse transgenic models, genetic backgrounds and diets. First, we  
153 investigated B6D2/F1 transgenic mice with *Lipe* haploinsufficiency fed with 60% high fat  
154 diet<sup>13</sup>. Compared to obese wild type littermates, the mice showed no differences in body  
155 weight and fat mass (**Supplementary Fig. 2a,b**). During euglycemic hyperinsulinemic clamp,  
156 the glucose infusion rate tended to increase (**Fig. 2a**) while there was no change in glucose  
157 rate of disappearance in obese *Lipe* haploinsufficient mice compared with wild type  
158 littermates (**Fig. 2b**). Insulin-mediated suppression of hepatic glucose production was  
159 improved (**Fig. 2c**). In a second cohort of B6D2/F1 mice fed 45% high fat diet, insulin  
160 tolerance was improved while body weight was not modified in *Lipe* haploinsufficient mice  
161 compared with wild type littermates (**Fig. 2d, Supplementary Fig. 2c**). In a third cohort of  
162 C57BL/6J mice fed 60% high fat diet, we confirmed enhanced insulin sensitivity in *Lipe*  
163 haploinsufficient mice as determined by quantitative insulin-sensitivity check index  
164 (QUICKI) (**Fig. 2e**). Adipose *Elovl6* gene expression was higher in mice with diminished  
165 HSL expression (**Fig. 2f**). As in human adipocytes (**Fig. 1j, Supplementary Fig 1h**), the  
166 induction was more pronounced for *Elovl6* than for other lipogenic genes (**Supplementary**  
167 **Fig. 2d**).

168 To generate a mouse model with HSL knock down in adipose tissue and unaltered expression  
169 in liver, we produced B6D2/F1 mice with zinc finger nuclease-mediated deletion of exon B  
170 (**Supplementary Fig. 2e**). The promoter upstream of exon B governs HSL expression in fat  
171 cells<sup>18</sup>. *Lipe*<sup>exonB<sup>-/-</sup></sup> mice showed decreased expression of HSL in adipose tissue (**Fig. 2g,h**). In  
172 liver, the low levels of HSL which are mainly composed of exon A-containing transcripts,  
173 were not modified (**Supplementary Fig. 2f,g**). *Lipe*<sup>exonB<sup>-/-</sup></sup> mice fed high fat diet showed  
174 improved glucose tolerance (**Fig. 2i**) without alteration of body weight (**Supplementary Fig.**  
175 **2h**). Adipose *Elovl6* gene expression was higher in these mice compared to wild type

176 littermates (**Fig. 2j**). Pharmacological inhibition of HSL had positive effect in C57BL/6J  
177 mice. Chronic treatment with a specific inhibitor of HSL did not alter body weight  
178 (**Supplementary Fig. 2i**) but resulted in increased QUICKI (**Supplementary Fig. 2j**) and  
179 higher induction of adipose *Elovl6* gene expression (**Supplementary Fig. 2k**). Therefore,  
180 both genetic and pharmacologic inhibition of HSL results in improved insulin sensitivity and  
181 enhanced *Elovl6* expression in adipose tissue in vivo.

182  
183 **Adipose ELOVL6 has a positive effect on insulin signaling and is associated with insulin**  
184 **sensitivity in vitro and in vivo**

185 To determine whether ELOVL6 was involved in the improvement of insulin signaling when  
186 fat cell HSL expression is diminished, we performed siRNA-mediated knockdown of  
187 ELOVL6 in human adipocytes. Gene silencing led to a significant decrease in *ELOVL6*  
188 mRNA level and activity (**Supplementary Fig. 3a,b**). The increases in IRS1 (**Fig. 3a**) and  
189 AKT (**Fig. 3b, Supplementary Fig. 3c**) phosphorylation observed in HSL-deficient  
190 adipocytes were abrogated following concomitant gene silencing of ELOVL6. To assess the  
191 importance of *Elovl6* on adipose tissue insulin signaling in vivo, a bolus of insulin was  
192 injected to wild type and *Elovl6* null mice of similar body weights prior to collection and  
193 analyses of fat pads (**Supplementary Fig. 3d,e**). In agreement with in vitro data in human  
194 adipocytes, insulin-stimulated Akt phosphorylation was decreased in adipose tissue of *Elovl6*  
195 null mice (**Fig. 3c**). These results reveal a strong link between ELOVL6 and insulin signaling  
196 in fat cells and identify ELOVL6 as the mediator of the beneficial effects of HSL inhibition.

197 The relationship between adipose tissue ELOVL6 and insulin sensitivity was further explored  
198 in mouse models and clinical cohorts. In mice fed high fat diet, the C57BL/6J strain showed  
199 higher insulin tolerance than DBA/2J strain (**Fig. 3d, Supplementary Fig. 3f**). The better  
200 insulin action in C57BL/6J mice was accompanied by higher induction of adipose tissue  
201 *Elovl6* gene expression during refeeding (**Fig. 3e**). In humans, *ELOVL6* gene expression was

202 first measured in visceral adipose tissue from lean insulin-sensitive individuals and obese  
203 patients with metabolic syndrome, the latter being characterized by higher body mass index  
204 and lower glucose disposal rate measured during euglycemic hyperinsulinemic clamp (**Fig. 3f**,  
205 **Supplementary Fig. 3g**). Adipose tissue *ELOVL6* mRNA levels were lower in insulin  
206 resistant individuals (**Fig. 3g**). Additional evidence was provided by a longitudinal study. In  
207 morbidly obese subjects, the weight loss observed two years after bariatric surgery  
208 (**Supplementary Fig. 3h**) was associated with an improvement in insulin sensitivity  
209 estimated by euglycemic hyperinsulinemic clamp-derived M value (**Fig. 3h**) and an increase  
210 in subcutaneous adipose *ELOVL6* mRNA level (**Fig. 3i**). A strong positive correlation was  
211 found between *ELOVL6* mRNA levels in subcutaneous fat and M value (**Supplementary Fig.**  
212 **3i**). Taken together, both murine and human data show a positive association between adipose  
213 *ELOVL6* expression and insulin sensitivity in vivo.

214

### 215 **ELOVL6 positive effect on insulin signaling is mediated by oleic acid content in PL and** 216 **plasma membrane fluidity**

217 We sought to identify the mechanisms by which ELOVL6 improves insulin signaling.  
218 Considering that the enzyme catalyzes critical steps in FA synthesis (**Supplementary Fig.**  
219 **1d**), we investigated the direct contribution of ELOVL6 on the changes in fat cell FA  
220 composition. In TG (**Fig. 4a**) and PL (**Fig. 4b**) of human adipocytes, diminished ELOVL6  
221 expression led to an increase in palmitic acid and palmitoleic acid at the expense of oleic acid.  
222 Analyses of these FAs were then performed on each class of PL. ELOVL6-deficient  
223 adipocytes showed a decrease in the proportion of oleic acid (e.g., 36:2) and an increase in the  
224 proportion of palmitic acid (e.g., 32:0) and palmitoleic acid (e.g., 32:2) in  
225 phosphatidylcholines and phosphatidylethanolamines (**Supplementary Fig. 4a-d**). In  
226 phosphatidylinositides, there was a decrease in 36:2 whereas no change was observed for

227 phosphatidylserines. These findings were in agreement with in vivo data, where the lack of  
228 *Elovl6* in mouse adipose tissue resulted in increased palmitic and palmitoleic acid and  
229 decreased oleic acid contents in adipose tissue (**Supplementary Fig. 4e**). The changes in FA  
230 composition of PL suggest potential modification in plasma membrane properties. As  
231 ELOVL6 mediates the positive effect of HSL gene silencing on insulin signaling (**Fig. 3a,b**,  
232 **Supplementary Fig. 3c**), we determined whether this effect was dependent on oleic acid.  
233 There are two enzymatic steps between palmitic acid and oleic acid (**Supplementary Fig.**  
234 **1d**). The first is the elongation of palmitic acid into stearic acid catalyzed by ELOVL6 and the  
235 second is the desaturation of stearic acid into oleic acid catalyzed by SCD, a highly active  
236 process in fat cells<sup>19-22</sup>. To investigate the respective contribution of the two steps, specific  
237 inhibitors were used. Treatment of human adipocytes with an inhibitor of ELOVL6<sup>23</sup> resulted  
238 in the expected changes in FA composition with a decrease of the C18/C16 FA ratio (**Fig. 4c**).  
239 Concordant with data obtained using gene silencing (**Fig. 3b**), pharmacological inhibition of  
240 ELOVL6 abrogated the enhancement of insulin-induced AKT phosphorylation observed in  
241 HSL-deficient adipocytes (**Fig. 4d**). A specific SCD inhibitor<sup>24</sup> decreased C16 and C18 FA  
242 desaturation (**Fig. 4e**) and had the same effect as the ELOVL6 inhibitor on AKT  
243 phosphorylation (**Fig. 4f**). Our data suggest that SCD is necessary but does not play a rate-  
244 limiting role as does ELOVL6 in the improvement of insulin signaling induced by HSL  
245 inhibition. Accordingly, *SCD* mRNA levels are much higher than *ELOVL6* mRNA levels in  
246 human adipocytes (**Supplementary Fig. 4f**). In additional experiments, the content of oleic  
247 acid in PL was directly modified by incubation of adipocytes with the FA (**Fig. 4g**). Exposure  
248 of HSL and ELOVL6 double deficient adipocytes to oleic acid rescued insulin-induced AKT  
249 phosphorylation to levels comparable with that observed in fat cells with diminished HSL  
250 expression (**Fig. 4h**). Therefore, the beneficial role of ELOVL6 on insulin signaling in human  
251 adipocytes is mediated by modulation of oleic acid content.

252 The composition of FA in PL may influence insulin signal transduction through modification  
253 of plasma membrane properties<sup>19,25,26</sup>. To determine the consequence of ELOVL6-mediated  
254 changes in PL FA composition on plasma membrane fluidity, overexpression of ELOVL6 in  
255 human adipocytes was achieved using an adenoviral vector (**Supplementary Fig. 4g,h**). The  
256 resulting increase in C18/C16 FA ratio (**Fig. 4i**) was associated with an increase in insulin-  
257 induced IRS1 phosphorylation (**Fig. 4j**). Fluorescence recovery after photobleaching (FRAP)  
258 data were analyzed in cells overexpressing ELOVL6 which plasma membrane glycolipids  
259 were labeled by fluorescent cholera toxin subunit B (**Fig. 4k**). Comparison of the mobile  
260 fractions (**Fig. 4l**) revealed an increase in plasma membrane lateral mobility of cholera toxin-  
261 bound glycolipids in ELOVL6-overexpressing adipocytes. Collectively, the data suggest that  
262 enhanced adipocyte ELOVL6 activity increases the proportion of oleic acid in PL and  
263 positively influences insulin signaling through modulation of plasma membrane fluidity.

264

265 **The glucose-responsive transcription factor ChREBP mediates the beneficial effect of**  
266 **diminished HSL expression through ELOVL6 induction**

267 ELOVL6 is a direct transcriptional target of ChREBP<sup>27</sup>. Adipose ChREBP is a major  
268 determinant of systemic insulin action on glucose metabolism<sup>28</sup>. Therefore, the direct  
269 contribution of ChREBP to HSL gene silencing-mediated improvement of glucose  
270 metabolism in human adipocytes was evaluated using RNA interference (**Supplementary**  
271 **Fig. 5a**). During dual knockdown of HSL and ChREBP, the beneficial effects on glucose  
272 metabolism observed in adipocytes with low HSL expression were diminished. Glucose  
273 transport was decreased to control levels and the induction of *de novo* lipogenesis observed in  
274 cells with single HSL knockdown was lowered in adipocytes with dual knockdown of HSL  
275 and ChREBP (**Fig. 5a,b**). A similar pattern was observed for glucose and acetate carbon  
276 incorporation into FA (**Fig. 5b,c**) showing that the upregulation of FA synthesis resulted not

277 only from increased glucose uptake but also from specific induction of *de novo* lipogenesis.  
278 ChREBP gene silencing also mitigated the increase in insulin-induced IRS1 and AKT  
279 phosphorylation (**Fig. 5d,e**). These results indicate that ChREBP is involved in the  
280 improvement of glucose metabolism and insulin signaling induced by HSL downregulation.  
281 Similarly to what had been observed for ELOVL6 knock down (**Fig. 4a,b**), ChREBP gene  
282 silencing led to an increase in palmitic acid and palmitoleic acid and a decrease of oleic acid  
283 (**Fig. 5f,g**). Accordingly, ChREBP gene silencing potently suppressed *ELOVL6* gene  
284 expression but had weak or no effect on other lipogenic genes suggesting that ELOVL6 is a  
285 preferential target of ChREBP in human fat cells (**Fig. 5h**). The involvement of ChREBP in  
286 adipose *Elovl6* gene expression was confirmed in vivo. In adipose tissue of *Mlxipl* null mice  
287 (**Supplementary Fig. 5b**), *Elovl6* was the lipogenic gene which expression was the most  
288 severely impaired (**Fig. 5i**).

289 Two isoforms of ChREBP have been identified. ChREBP $\alpha$ , which transcriptional activity is  
290 regulated by glucose, and ChREBP $\beta$ , a transcriptionally superactive and unstable isoform  
291 which is a direct transcriptional target of ChREBP $\alpha$  <sup>28,29</sup>. We characterized the human  $\beta$ -  
292 specific exon of *MLXIPL* which extends 29 deoxynucleotides 3' of its mouse counterpart <sup>28</sup>  
293 (**Supplementary Fig. 5c**). In human adipocytes with siRNA-mediated knock down of HSL,  
294 the levels of *ChREBP* transcripts, notably the  $\beta$  isoform, were increased (**Fig. 5j**).

295 Recruitment of ChREBP on the functional carbohydrate response element (ChoRE) in the  
296 *ELOVL6* promoter was investigated using chromatin immunoprecipitation assays <sup>27</sup>. In  
297 hMADS adipocytes, more binding events were detected on the *ELOVL6* ChoRE than on  
298 positive control regions in *RORC* and *TXNIP*, a well characterized target of ChREBP  
299 (**Supplementary Fig. 5d**). ChREBP recruitment onto the *ELOVL6* promoter was markedly  
300 enhanced in HSL-deficient compared to control adipocytes (**Fig. 5k**).

301 Interestingly, *ELOVL6* was strongly associated with *ChREBPβ* gene expression in human  
302 hMADS adipocytes (**Fig. 5l**) and human differentiated primary preadipocytes  
303 (**Supplementary Fig. 5e**). Albeit less potent, a positive correlation was also found between  
304 *ELOVL6* and *ChREBPα* (**Supplementary Fig. 5f**). Similarly, a highly significant correlation  
305 between *ELOVL6* and *ChREBPβ* was observed in human subcutaneous adipose tissue  
306 samples (**Fig. 5m**). Short term elevation in plasma glucose and insulin levels during a  
307 hyperglycemic hyperinsulinemic clamp led to a pronounced induction of adipose *ChREBPβ*  
308 (**Fig. 5n**) and *ELOVL6* (**Fig. 5o**) gene expression, illustrating the importance of glucose flux  
309 into the fat cells in the control of *ELOVL6* expression in humans. Altogether, our results show  
310 that ChREBPβ mediates the effect of HSL deficiency on glucose metabolism and insulin  
311 signaling through transcriptional activation of *ELOVL6*.

312

### 313 **HSL modifies ChREBP activity in fat cells through protein-protein interaction**

314 As HSL catalyzes one of the rate-limiting steps in fat cell TG hydrolysis, we investigated  
315 whether lipolysis per se contributed to the induction of ChREBP. Several lines of evidence  
316 suggest that this is not the case. In the culture conditions used to study *de novo* lipogenesis,  
317 the release of glycerol and FA in the culture medium was low and was not influenced by HSL  
318 gene silencing (**Supplementary Fig. 6a,b**). Adipocytes were then treated with triacsin C, a  
319 potent inhibitor of long chain fatty acyl CoA synthetase<sup>14</sup>. If FAs were involved, enhanced  
320 FA levels due to blockade of FA re-esterification should influence *ChREBP* and *ELOVL6*  
321 induction in adipocytes with HSL knock down. However, the upregulation of *ChREBP*  
322 isoform and *ELOVL6* mRNA was not influenced by the treatment (**Supplementary Fig. 6c-**  
323 **e**). These data show that the lipolytic activity of HSL does not contribute to the induction of  
324 ChREBP.

325 These findings led us to hypothesize that physical interaction between HSL and ChREBP may  
326 influence ChREBP activity. HEK293 cells were transfected with vectors expressing HSL and  
327 ChREBP $\alpha$  with FLAG epitope tag <sup>30</sup>. Immunoprecipitation of cell lysates with anti-FLAG  
328 IgG and immunoblotting with anti-HSL antibody showed that HSL co-immunoprecipitated  
329 with ChREBP $\alpha$  (**Supplementary Fig. 6f**). Co-immunoprecipitation was observed using  
330 FLAG-ChREBP immobilized on magnetic beads and recombinant HSL (**Fig. 6a**). Surface  
331 plasmon resonance assays supported a direct binding between ChREBP $\alpha$  and HSL (**Fig. 6b**).  
332 Interaction between endogenous proteins in adipocytes was shown through  
333 immunoprecipitation with anti-ChREBP and anti-HSL antibodies (**Fig. 6c, Supplementary**  
334 **Fig. 6g**). In line with the lack of effect on *de novo* lipogenesis (**Supplementary Fig. 1k,l**),  
335 ATGL displayed no interaction with ChREBP $\alpha$  further indicating that HSL interaction with  
336 ChREBP $\alpha$  is independent lipolysis and specific to this neutral lipase (**Fig. 6c**).  
337 Furthermore, interaction of HSL with ChREBP $\alpha$  was shown using in situ proximity ligation  
338 assays using a pair of primary antibodies raised in two different species and a pair of  
339 secondary antibodies coupled to oligodeoxynucleotides <sup>31</sup>. Specific and robust fluorescence  
340 signals were observed in the cytosol of fat cells from subcutaneous adipose tissue  
341 (**Supplementary Fig. 6h**). Such signals were also seen in differentiated hMADS adipocytes  
342 (**Supplementary Fig. 6i**). Little signal was detected in undifferentiated fibroblasts which do  
343 not express HSL. Negative controls using incomplete sets of antibodies and assays using anti-  
344 ATGL and anti-AKT combined with anti-ChREBP antibodies supported specificity of the  
345 interaction (**Supplementary Fig. 6j,k**). Human HepG2 hepatocytes which express significant  
346 level of ChREBP but minute amounts of HSL showed few fluorescent spots (**Supplementary**  
347 **Fig. 6l,m**). Respective expression of ChREBP $\alpha$  and HSL in mouse tissues is coherent with a  
348 fat-specific interaction of the two proteins (**Supplementary Fig. 6n**). The data suggest that  
349 ChREBP $\alpha$  interaction with HSL is specific to fat cells.



350 In mouse adipose tissue, co-immunoprecipitation between HSL and ChREBP was diminished  
351 in *Lipe* haploinsufficient mice (**Fig. 6d**). In human adipocytes, HSL binding to ChREBP $\alpha$  was  
352 reduced in cytoplasm when HSL expression was diminished using siRNA (**Fig. 6e**,  
353 **Supplementary Fig. 7a**). This resulted in modification of ChREBP cellular distribution.  
354 Compared to control cells, adipocytes with low HSL expression showed higher  
355 immunofluorescence of ChREBP in nuclei indicating that ChREBP $\alpha$  nuclear translocation is  
356 facilitated when interaction with HSL is diminished (**Fig. 6f**). Subcellular fractionation  
357 confirmed an increased nuclear translocation in human adipocytes and mouse adipose tissues  
358 with low HSL expression whereas no significant differences was observed in the cytosolic  
359 fraction (**Fig. 6g,h, Supplementary Fig. 7b,c**). In mice, there was no difference in ChREBP $\alpha$   
360 protein content in fat pads of the two genotypes (**Supplementary Fig. 7d**). To evaluate the  
361 effect of HSL on ChREBP $\alpha$  transcriptional activity, HEK293 cells were transfected with a  
362 vector containing the luciferase reporter gene under the control of a promoter containing  
363 functional ChoREs<sup>32</sup>. Promoter activity increased when cells expressed ChREBP $\alpha$  and  
364 decreased when cells co-expressed increasing amounts of HSL (**Fig. 6i, Supplementary Fig.**  
365 **7e**). The data suggest that HSL binds to ChREBP $\alpha$  and sequesters the transcription factor in  
366 the cytoplasm. Upon decrease of HSL expression, HSL-ChREBP interaction is diminished,  
367 ChREBP $\alpha$  nuclear translocation is facilitated and its transcriptional activity is enhanced as  
368 shown here in reporter gene assays and above in chromatin immunoprecipitation analysis  
369 (**Fig. 5k**).

370 When HEK293 cells expressing HSL and ChREBP were treated with a HSL inhibitor, less  
371 interaction between HSL and ChREBP was observed (**Supplementary Fig. 7f**). In human  
372 adipose tissue, we previously identified a short form of HSL produced by in-frame skipping  
373 of exon 6 (**Supplementary Fig. 2e**)<sup>33,34</sup>. As exon 6 encodes the catalytic site Serine, HSL  
374 short form is devoid of enzymatic activity (**Supplementary Fig. 7g**). Expressed in HEK293

375 cells, HSL short form retained the capacity to bind ChREBP (**Fig. 6j**). An adenovirus  
376 expressing HSL short form was used to transduce human adipocytes transfected with control  
377 or *LIPE* siRNA (**Supplementary Fig. 7h**). The induction of *ELOVL6* in adipocytes with  
378 diminished levels of HSL was blunted when HSL short form was expressed (**Fig. 6k**). A  
379 similar pattern was observed for other *de novo* lipogenesis gene expression (**Supplementary**  
380 **Fig. 7i**). The catalytically inactive form also reduced the increase in IRS1 phosphorylation  
381 mediated by HSL down regulation (**Fig. 6l**). Of note, the improvement of fat cell insulin  
382 signaling was observed with no change in amount of ChREBP $\alpha$  protein in adipocytes  
383 expressing HSL-S and in the absence of correlation between HSL and ChREBP $\alpha$  levels.  
384 (**Supplementary Fig. 7j,k**). Altogether, our data suggest that HSL plays an important role  
385 besides the hydrolysis of lipids in fat cells, the repression of ChREBP activity via direct  
386 interaction with the transcription factor (**Supplementary Fig. 8**).

387  
388

## Discussion

389 Considering the soaring incidence of diseases characterized by an insulin resistance state,  
390 there is a lack of drugs acting on adipose tissue. Its specialized cells, the adipocytes, have  
391 great potential to be therapeutically targeted<sup>35</sup>. Partial inhibition of the fat cell neutral lipase,  
392 HSL, alleviates insulin resistance without increasing body weight, two essential requirements  
393 for therapeutic interventions<sup>13</sup> (and present work). Evolution of plasma fatty acid level and  
394 variation in insulin sensitivity was dissociated in this model. Here, we deciphered the  
395 mechanisms behind HSL inhibition-mediated improvement of glucose metabolism and  
396 identified interactions between prototypical metabolic pathways of the adipocyte. We show  
397 that, independently of lipolysis and the enzyme catalytic activity, HSL physically interacts  
398 with and inhibits the transcription factor ChREBP. ChREBP controls the FA elongase  
399 *ELOVL6* catalyzing a limiting step in oleic acid synthesis. The resulting increase in PL oleic  
400 acid content modifies plasma membrane properties and improves insulin signaling.

401 Adipose *de novo* lipogenesis is positively associated with systemic insulin sensitivity<sup>36,37</sup>. *De*  
402 *novo* lipogenesis is under the control of the glucose-responsive transcription factor, ChREBP.  
403 A positive association between insulin sensitivity and adipose ChREBP, notably, the  
404 transcriptionally superactive  $\beta$  isoform, has been reported<sup>16,28,38</sup>. In human adipocytes, we  
405 show that knockdown of ChREBP counteracts the beneficial effects of HSL gene silencing on  
406 insulin sensitivity. We identify the FA elongase ELOVL6 as the main target of ChREBP $\beta$  in  
407 HSL-deficient adipocytes. In humans, *ELOVL6* expression in fat was lower in insulin resistant  
408 than in insulin sensitive subjects in line with previous reports<sup>16,39</sup>. Of note, in monozygotic  
409 twin pairs discordant for type 2 diabetes, adipose *ELOVL6* is markedly lower in the affected  
410 twins<sup>40</sup>. Our results from bariatric surgery, a longitudinal intervention improving insulin  
411 control of glucose metabolism, also supported the tight link between adipose *ELOVL6* and  
412 insulin sensitivity.

413 ELOVL6 catalyzes a critical step in the elongation of C16 FA<sup>22,41</sup>. In adipocytes, enhanced  
414 ELOVL6 activity favored oleic acid synthesis while ELOVL6 knock down had the opposite  
415 effect. Diets rich in olive oil improve insulin sensitivity at adipocyte and whole-body levels  
416<sup>42,43</sup>. This effect may contribute to the decreased incidence of type 2 diabetes in patients at risk  
417 fed Mediterranean diets<sup>43</sup>. At the cellular level, monounsaturated fatty acids have been  
418 reported to protect from the damaging effect of palmitic acid on insulin signaling<sup>19,26</sup>. As  
419 ELOVL6 induced an increase of oleic acid in major classes of PL, we postulated that it may  
420 alter plasma membrane fluidity owing to its conformational plasticity<sup>25,44,45</sup>. The plasma  
421 membrane lateral mobility of glycolipids was increased in fat cells overexpressing ELOVL6.  
422 We therefore propose that ELOVL6-mediated increase in PL oleic acid content improves fat  
423 cell insulin signaling through alteration of plasma membrane properties. In mice, *Elovl6*  
424 deficiency impaired white adipose tissue insulin signaling whereas the opposite or lack of

425 alteration have previously been reported in the liver suggesting tissue-specific differences in  
426 ELOVL6-mediated modulation of insulin action<sup>41,46</sup>.

427 The role of HSL in human fat cell lipolysis is well established<sup>14,47</sup>. HSL is a multifunctional  
428 enzyme with a broad range of substrates. Besides tri-, di- and monoglycerides, HSL is able to  
429 hydrolyze other esters, such as cholesteryl and retinyl esters<sup>48</sup>. As ChREBP activity is  
430 influenced by metabolites and other transcription factors in liver, it could be postulated that  
431 products of HSL enzymatic activity directly or indirectly influence ChREBP-mediated  
432 modulation of gene transcription<sup>29</sup>. However, although we cannot rule out that, in some  
433 conditions, upregulation of ChREBP $\alpha$  protein expression partially contributes to the  
434 phenotype of adipocytes depleted in HSL, we bring a solid body of evidence that physical  
435 interaction between HSL and ChREBP $\alpha$  controls the intracellular location and activity of the  
436 transcription factor in fat cells. ATGL which catalyzes the first step in adipose tissue lipolysis  
437 does not interact with ChREBP and does not modulate *de novo* lipogenesis gene expression.  
438 Moreover, using a short inactive form of HSL lacking the catalytic site Serine, we could show  
439 that the catalytic activity of HSL is dispensable for the interaction with ChREBP and  
440 ChREBP-mediated effect on *ELOVL6* expression and insulin signaling. Noteworthy, our data  
441 provide a function to this naturally occurring form expressed in human adipose tissue<sup>33</sup>. A  
442 specific HSL inhibitor was able to diminish the interaction between HSL and ChREBP and  
443 enhance adipose *Elovl6* expression in mice. It may be hypothesized that the inhibitor binding  
444 to the catalytic pocket induces conformational change partially disrupting HSL-ChREBP  
445 interaction. The data suggest that small molecules may be designed and used to disrupt the  
446 interaction. Reducing the interaction between HSL and ChREBP favors ChREBP nuclear  
447 translocation and its transcriptional activity. This pathway provides a molecular basis to the  
448 differential control of *de novo* lipogenesis in liver and adipose tissue. ChREBP is involved in  
449 the regulation of *de novo* lipogenesis in the two tissues<sup>29,49</sup>. However, the pathway is

450 generally considered as detrimental in the liver as it is activated during the development of  
451 fatty liver disease whereas it is seen as beneficial in adipose tissue as the link with insulin  
452 sensitivity has been shown both in clinical studies and in mouse models<sup>50</sup>. HSL is expressed  
453 at much higher level in fat cells than in hepatocytes. Accordingly, interaction between HSL  
454 and ChREBP is not found in human hepatocytes. Alleviation of HSL-mediated inhibition of  
455 ChREBP activity may constitute a fat cell-specific mechanism to enhance *de novo* lipogenesis  
456 and insulin signaling.

457 To conclude, our work identifies a pathway critical for optimal insulin signaling in fat cells  
458 which links the neutral lipase HSL to the glucose-responsive transcription factor ChREBP and  
459 its target gene, the FA elongase, ELOVL6. This constitutes a unique example of an enzyme  
460 involved in lipid metabolism which independently of its enzymatic activity inhibits the  
461 transcriptional activity of a glucose-responsive transcription factor through protein-protein  
462 interaction. Inhibition of the HSL-ChREBP interaction may constitute an adipose-specific  
463 strategy to reduce insulin resistance.

464

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480

481 AUTHOR CONTRIBUTIONS

482 P.M. and M.H. share first authorship. P.M. and M.Ho. performed the majority of in vitro  
483 experiments and analyzed data with the contribution of A.Mai., C.G., F.B., B.M., E.R.,  
484 P.D.D., V.Sr., V.B., D.B., M.M., C.L., L.L., F.L. and M.Ha. P.M., M.Ho., E.Mo., G.T., S.V.,  
485 L.M., S.G., B.M.-R., T.S., H.G., C.H., A.V.P. and C.P. performed and analyzed in vivo data  
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487 M.R., N.V. and H.V. performed and analyzed in vivo data in human clinical studies. S.C.-B.,  
488 S.V. and J.B.-M. analyzed lipidomics data. A.Maz. and M.Z. performed and analyzed FRAP

489 experiments. B.P., C.M., N.V., S.H. and H.V. interpreted the data. P.M., M.Ho. and D.L.  
490 conceived the study, interpreted the data and wrote the manuscript. D.L. supervised the study.

491

#### 492 COMPETING INTERESTS STATEMENT

493 T.S. is an employee of Physiogenex. M.H. and S.H. are employees of AstraZeneca. The other  
494 authors declare no competing financial and non-financial interests.

495

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- 625

626 FIGURE LEGENDS

627 **Figure 1.** Reduced HSL expression promotes glucose metabolism and insulin signaling in  
628 human adipocytes. Experiments were carried out in control (white bars, siCTR) and HSL-  
629 deprived (grey bars, siHSL) hMADS adipocytes. **(a-g)** Adipocytes were analyzed in basal (-)  
630 and insulin-stimulated (+, 100nM) conditions. **(a)** Glucose transport using radiolabelled 2-  
631 deoxyglucose (n=12 biologically independent samples per group) (Insulin stimulation:  
632 P<0.0001). **(b)** Glucose oxidation using radiolabelled glucose (n=10 biologically independent  
633 samples per group) (Insulin stimulation: P=0.0015). **(c)** *de novo* lipogenesis using  
634 radiolabelled glucose (n=10 biologically independent samples per group) (Insulin stimulation:  
635 P<0.0001). **(d-g)** Insulin signaling evaluated by activating phosphorylation of IRS1 (pY612)  
636 (n=7 biologically independent samples per group) (Insulin stimulation: P=0.0033) **(d)** AKT  
637 (pS473) (n=5 biologically independent samples per group) (Insulin stimulation: P=0.0005)  
638 **(e)**, AKT (pT308) (n=8 biologically independent samples per group) (Insulin stimulation:  
639 P=0.0201) **(f)** and AS160 (pT642) (n=4 biologically independent samples per group) (Insulin  
640 stimulation: P=0.0726) **(g)**. Size markers (in kDa) are shown on illustrative Western blot  
641 panels. **(h, i)** Fatty acid composition in triglycerides (TG) **(h)** and phospholipids (PL) **(i)** (n=8  
642 biologically independent samples per group). **(j)** mRNA levels of lipogenic enzymes (n=6  
643 biologically independent samples per group). Data are mean  $\pm$ sem. Statistical analysis was  
644 performed using two-way ANOVA with Bonferroni's post hoc tests **(a-g)**, paired Student's t  
645 test **(h, i)** and Wilcoxon's test **(j)**. Statistical tests were two-sided. \*P<0.05, \*\*P<0.01,  
646 \*\*\*P<0.001 compared to control.

647

648 **Figure 2.** HSL inhibition is associated with increased insulin sensitivity and adipose tissue  
649 ELOVL6 expression *in vivo*. **(a-f)** Experiments were carried out in wild type (WT, white bars)  
650 and HSL haploinsufficient (*Lipe*<sup>+/-</sup>, grey bars) mice. **(a-c)** Glucose infusion rate (GIR) **(a)** post

651 insulin glucose rate of disappearance (Glucose Rd) **(b)** and hepatic glucose production (HGP)  
652 **(c)** during euglycemic-hyperinsulinemic clamp in B6D2/F1 mice fed 60% high fat diet for 3  
653 months (WT n=7 animals, *Lipe*<sup>+/-</sup> n=6 animals). **(d)** Plasma glucose concentration during an  
654 insulin tolerance test in B6D2/F1 mice fed 45% high fat diet for 3 months (n=12 animals per  
655 group). **(e)** QUICKI and **(f)** mRNA level of *Elovl6* in response to refeeding in gonadal  
656 adipose tissue (n=8 animals per group) in C57BL/6J mice fed 60% high fat diet for 3 months  
657 (n=8 animals per group). **(g-j)** Experiments were carried out in wild type (WT, white bars)  
658 and in mice with zinc finger nuclease-mediated deletion of *Lipe* exon B which promoter  
659 drives HSL expression in fat cells (*Lipe*<sup>exonB-/-</sup>, grey bars). **(g)** mRNA levels of transcripts  
660 containing different exons encoding HSL in inguinal adipose tissue (n=12 animals per group).  
661 **(h)** Western blot analysis of adipose tissue HSL protein content (10 µg total protein) (WT n=7  
662 animals; *Lipe*<sup>exonB-/-</sup> n =5 animals). GAPDH was used as Western blot loading control. Size  
663 markers (in kDa) are shown on illustrative Western blot panel. **(i)** Plasma glucose  
664 concentration and area under the curve (AUC) during a glucose tolerance test (WT n=7  
665 animals, *Lipe*<sup>exonB-/-</sup> n=5 animals) in mice fed 60% high fat diet for 3 weeks and **(j)** mRNA  
666 level of adipose tissue *Elovl6* (WT n=5 animals, *Lipe*<sup>exonB-/-</sup> n=6 animals). Data are mean  
667 ±sem. Statistical analysis was performed using Mann and Whitney's test **(a-c, h-j)**, unpaired  
668 Student's t test **(e-g)** or two-way ANOVA with Bonferroni's post-hoc tests **(d)**. Statistical  
669 tests were two-sided. \*P<0.05 compared to control.

670

671 **Figure 3.** ELOVL6 has a positive effect on insulin signaling in adipocytes. **(a, b)** Experiments  
672 were carried out in control (white bars, siCTR), single HSL (grey bars, siHSL), single  
673 ELOVL6 (light orange bars, siELOVL6) or dual HSL/ELOVL6-deprived (dark orange bars,  
674 siHSL/siELOVL6) hMADS adipocytes in basal (-) and insulin-stimulated (+, 100nM)  
675 conditions. Insulin signaling evaluated by activating phosphorylation of IRS1 (pY612) (n=7

676 biologically independent samples per group) (Insulin stimulation:  $P=0.0238$ ) (a) and AKT  
677 (pS473) (n=7 biologically independent samples per group) (Insulin stimulation:  $P<0.0001$ )  
678 (b). (c) Insulin signaling evaluated by activating phosphorylation of AKT (pS473) in wild  
679 type (WT, white bars, n=4 animals) and *Elovl6* null (*Elovl6*<sup>-/-</sup>, light orange bars, n=3 animals)  
680 mice injected with a bolus of insulin (Insulin stimulation:  $P<0.0001$ ). Size markers (in kDa)  
681 are shown on illustrative Western blot panels. (d, e) Plasma glucose concentration during an  
682 insulin tolerance test (n=9 animals per group) (d) and gonadal adipose tissue *Elovl6* mRNA  
683 levels in response to refeeding (e) in DBA/2J (white bars, n=6 animals) and C57Bl/6J (light  
684 green bars, n=5 animals) mice. (f, g) Glucose disposal rate (GDR) (f) and mRNA level of  
685 *ELOVL6* in visceral adipose tissue (g) from lean healthy (LE, white bars, n=13 individuals)  
686 and obese women with metabolic syndrome (MS, light red bars, n=15 individuals). (h, i) M-  
687 value (h) and normalized *ELOVL6* mRNA level (i) in subcutaneous adipose tissue of obese  
688 women before and two years after bariatric surgery (n=14 individuals). Data are mean  $\pm$ sem.  
689 Statistical analysis was performed using paired (a, b, d) and unpaired (c) two way ANOVA  
690 with Bonferroni's post hoc tests, unpaired Student's t test (f), Mann and Whitney's test (e, g),  
691 and Wilcoxon's test (h, i). Statistical tests were two-sided. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$   
692 compared to control condition or other mouse strain. In cell experiments, <sup>§§</sup> $P<0.01$ ,  
693 <sup>§§§</sup> $P<0.001$  compared to HSL-deprived adipocytes.

694

695 **Figure 4.** Oleic acid content in PL and plasma membrane fluidity mediates ELOVL6 positive  
696 effect on insulin signaling. (a-h) Experiments were carried out in control (white bars, siCTR),  
697 single HSL (grey bars, siHSL), single ELOVL6 (light orange bars, siELOVL6) or dual  
698 HSL/ELOVL6-deprived (dark orange bars, siHSL/siELOVL6) hMADS adipocytes. (a, b)  
699 Fatty acid composition in TG (a) and PL (b) (n=6 biologically independent samples per  
700 group). (c-f) hMADS adipocytes were treated with vehicle (DMSO), 1 $\mu$ M of ELOVL6



701 inhibitor (ELOVL6i) or 75nM of SCD inhibitor (SCDi) for 48h. Fatty acid ratios (n=6 for  
702 ELOVL6i and n=5 for SCDi biologically independent samples per group) (**c,e**) and insulin  
703 signaling evaluated by activating phosphorylation of AKT (pS473) in basal (-) and insulin-  
704 stimulated conditions (+,100nM) (n=4 biologically independent samples per group for  
705 ELOVL6i and SCDi) (Insulin stimulation:  $P<0.0001$ ) (**d,f**). DMSO-treated adipocyte values  
706 are common to panels **d** and **f** and, **Supplementary Fig. 1e**. (**g, h**) hMADS were treated with  
707 vehicle (V), 100 $\mu$ M (O100) or 500 $\mu$ M (O500) of oleic acid for 48h. Oleic acid levels in PL  
708 (n=5 biologically independent samples per group) (**g**) and insulin signaling evaluated by  
709 activating phosphorylation of AKT (pS473) (n=5 biologically independent samples per group)  
710 in basal (-) or insulin-stimulated (+, 100nM) conditions (Insulin stimulation:  $P=0.0003$ ) (**h**).  
711 For panels **d, f** and **h**, cropped images of vehicle and treatment lanes originate from the same  
712 blot. Size markers (in kDa) are shown on illustrative Western blot panels. (**i-l**) Experiments  
713 were carried out in control hMADS adipocytes expressing green fluorescent protein (GFP)  
714 (white bars, Adeno-CTR) or overexpressing human ELOVL6 and GFP (avocado bars, Adeno-  
715 ELOVL6). (**i, j**) Fatty acid ratio (n=8 biologically independent samples per group) (**i**) and  
716 activating phosphorylation of IRS1 (pY612) in basal (-) and insulin-stimulated conditions (+,  
717 100nM) (n=8 biologically independent samples per group) (Insulin stimulation:  $P<0.0001$ ) (**j**).  
718 (**k, l**) FRAP experiments using fluorescent cholera toxin B (Alexa555-CTxB) (n=5  
719 independent experiments). (**k**) Representative confocal microscope image showing GFP and  
720 Alexa455-CTxB at room temperature of a successfully transduced hMADS adipocyte. Scale  
721 bar, 50 $\mu$ m. (**l**) Calculated mobile fraction (white bar, n=17 analyzed cells; avocado bar, n=16  
722 analyzed cells). Data are mean  $\pm$ sem. Statistical analysis was performed using Wilcoxon's test  
723 (**a,b**), paired (**c-f, h, j**) and unpaired (**d**) two way ANOVA with Bonferroni's post hoc tests,  
724 Friedman's with Dunn's post hoc tests (**g**), paired Student's t test (**i**) and Mann and Whitney's  
725 test (**l**). Statistical tests were two-sided. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  compared to control.

726 <sup>\$\$</sup>P<0.01, <sup>\$\$\$</sup>P<0.001 compared to HSL-deprived adipocytes. <sup>#</sup>P<0.05, <sup>###</sup>P<0.01 compared to  
727 HSL- and ELOVL6-deprived adipocytes.

728

729 **Figure 5.** The glucose-sensitive transcription factor, ChREBP, mediates the beneficial effect  
730 of diminished HSL expression on glucose metabolism and insulin signaling in adipocytes. **(a-**  
731 **e)** Experiments were carried out in control (white bars, siCTR), single HSL (grey bars,  
732 siHSL), single ChREBP (light brown bars, siChREBP) or dual HSL/ChREBP-deprived (dark  
733 brown bars, siHSL/siChREBP) hMADS adipocytes in basal (-) and insulin-stimulated (+,  
734 100nM) conditions. **(a)** Glucose transport using radiolabelled 2-deoxyglucose (n=12  
735 biologically independent samples per group) (Insulin stimulation: P<0.0001). **(b, c)** *de novo*  
736 lipogenesis using radiolabelled glucose (n=9 biologically independent samples per group)  
737 (Insulin stimulation: P=0.0002) **(b)** or radiolabelled acetate (n=6 biologically independent  
738 samples per group) (Insulin stimulation: P=0.0014) **(c)**. **(d, e)** Insulin signaling evaluated by  
739 activating phosphorylation of IRS1 (pY612) (n=8 biologically independent samples per  
740 group) (Insulin stimulation: P=0.0245) **(d)** and AKT (pS473) (n=8 biologically independent  
741 samples per group) (Insulin stimulation: P<0.0001) **(e)**. Size markers (in kDa) are shown on  
742 illustrative Western blot panels. **(f-h)** Experiments were carried out in control (white bars,  
743 siCTR) and ChREBP-deprived (light brown bars, siChREBP) hMADS adipocytes. **(f, g)** Fatty  
744 acid composition in TG **(f)** and PL **(g)** (n=8 biologically independent samples per group). **(h)**  
745 mRNA levels of lipogenic enzymes (n=6 biologically independent samples per group). **(i)**  
746 mRNA levels of lipogenic enzymes in inguinal adipose tissue of wild type (WT, white bars,  
747 n=7 animals) and *ChREBP* null mice (*Mlxipl*<sup>-/-</sup>, light brown bars, n=6 animals). **(j,k)**  
748 Experiments were carried out in control (white bars, siCTR) and HSL-deprived (grey bars,  
749 siHSL) hMADS adipocytes. **(j)** Induction of mRNA levels of *ChREBP* $\alpha$  and *ChREBP* $\beta$  (n=8  
750 biologically independent samples per group) and **(k)** ChREBP recruitment on *ELOVL6*

751 ChoRE (n=3 independent experiments). **(l, m)** Correlations between mRNA levels of  
752 *ELOVL6* and *ChREBPβ* in hMADS adipocytes (n=64 biologically independent samples) **(l)**  
753 and in human subcutaneous adipose tissue (n=31 individuals) **(m)**. **(n, o)** mRNA levels of  
754 *ChREBPβ* (n=7 biologically independent samples per group) **(n)** and *ELOVL6* (n=7  
755 biologically independent samples per group) **(o)** in human subcutaneous adipose tissue in  
756 basal condition or during hyperglycemic-hyperinsulinemic clamp. Data are mean ±sem.  
757 Statistical analysis was performed using paired two way ANOVA with Bonferroni post hoc  
758 tests **(a-e)**, paired Student's t test **(f, g, j)**, Wilcoxon's test **(h, n, o)**, Mann and Whitney's test  
759 **(i)** and linear regression **(l, m)**. Statistical tests were two-sided. \*P<0.05, \*\*P<0.01,  
760 \*\*\*P<0.001 compared to control. <sup>s</sup>P<0.05, <sup>ss</sup>P<0.01, <sup>sss</sup>P<0.001 compared to HSL-deprived  
761 adipocytes. <sup>#</sup>P<0.05, <sup>##</sup>P<0.01 compared to ChREBP-deprived adipocytes.

762

763 **Figure 6.** HSL inhibits ChREBP activity through protein-protein interaction. **(a)**  
764 Representative image of immunocomplexes between immobilized FLAG-ChREBPα and  
765 recombinant HSL (Rec.HSL) (n=3 independent experiments). **(b)** Representative surface  
766 plasmon resonance assay sensorgram showing the binding of HSL to ChREBP. Purified  
767 ChREBP (220RU) was first injected on a sensorchip with immobilized anti-ChREBP  
768 antibody. Following ChREBP binding, PR65α (no signal) and HSL (35RU) were  
769 consecutively injected (n=3 independent experiments). **(c)** Endogenous interaction between  
770 HSL and ChREBP in human adipocytes (n=3 biologically independent samples per group).  
771 Anti-ChREBP antibody was used for immunoprecipitation (IP). Normal Rabbit IgG antibody  
772 was used as negative control. **(d)** Endogenous interaction between HSL and ChREBP in white  
773 adipose tissue of *Lipe*<sup>+/-</sup> (+/-) and wild type (+/+) mice (n=4 animals per group). Anti-  
774 ChREBP antibody was used for immunoprecipitation. Rabbit IgG antibody was used as  
775 negative control. β-actin was used as Western blot loading control. **(e)** In situ proximity

776 ligation assays (red signals) performed with anti-HSL and anti-ChREBP antibodies (PLA  
777 HSL/ChREBP) and corresponding image under visible light (Phase) in control (siCTR) and  
778 HSL-deprived (siHSL) hMADS adipocytes. Nuclei were labelled in blue using DAPI.  
779 Representative image (6 independent experiments). Scale bars, 50 $\mu$ m. **(f)** Immunodetection of  
780 ChREBP (red) in control (siCTR) and HSL-deprived (siHSL) hMADS adipocytes.  
781 Representative image (4 independent experiments). Nuclei were labelled in blue using DAPI.  
782 Scale bars, 50 $\mu$ m. **(g)** ChREBP $\alpha$  protein levels in nuclear extracts from control (siCTR) and  
783 HSL-deprived (siHSL) hMADS adipocytes (n=8 biologically independent samples per  
784 group). **(h)** ChREBP $\alpha$  protein levels in nuclear extracts from white adipose tissue of wild type  
785 (WT, n=8 animals) and *Lipe*<sup>+/-</sup> (n=9 animals) mice. **(i)** Luciferase assays following  
786 transfection in HEK-293 cells of carbohydrate-responsive elements (ChoRE) fused to the  
787 luciferase gene along with expression vectors for ChREBP and HSL. ChoRE activity was  
788 measured in HEK-293 cells transfected with empty plasmid (pcDNA), ChREBP and different  
789 concentrations of HSL expression plasmids under low (5mM, G5) (n=6 biologically  
790 independent samples per group) and high (25mM, G25) glucose concentrations (n=4  
791 biologically independent samples per group). **(j)** HSL and ChREBP $\alpha$  immunocomplexes in  
792 HEK-293 cells transfected with empty plasmid (pcDNA3), FLAG-ChREBP, full length HSL  
793 (HSL) or short form HSL (HSL-S) expression plasmids. Anti-FLAG antibody was used for  
794 immunoprecipitation.  $\beta$ -actin was used as Western blot loading control (n=3 independent  
795 experiments) **(k,l)** Effect of overexpression of the short inactive form of HSL (HSL-S) in  
796 hMADS adipocytes. Experiments were carried out in control (siCTR, white bars) and HSL-  
797 deprived (siHSL, grey bars) hMADS adipocytes overexpressing green fluorescent protein  
798 (GFP, Ad-CTR) or the short inactive form of HSL (Ad-HSL-S). **(k)** mRNA levels of *ELOVL6*  
799 (n=10 biologically independent samples per group). **(l)** Activating phosphorylation of IRS1  
800 (pY612) in basal (-) and insulin-stimulated (+, 100nM) conditions (n=6 biologically

801 independent samples per group, Insulin stimulation:  $P < 0.0001$ ). Size markers (in kDa) are  
802 shown on illustrative Western blot panels. Data are mean  $\pm$  sem. Statistical analysis was  
803 performed using paired Student's t test (**g**), Mann and Whitney's test (**h**) or paired two-way  
804 ANOVA with Bonferroni's post-hoc tests (**i**, **k**, **l**). Statistical tests were two-sided. \* $P < 0.05$ ,  
805 \*\* $P < 0.01$  \*\*\* $P < 0.001$  compared to ChREBP condition (**i**) or control adipocyte (**l**). <sup>s</sup> $P < 0.05$ ,  
806 <sup>sss</sup> $P < 0.001$  compared to pcDNA condition (**i**) or siHSL/Ad-CTR adipocytes (**k**, **l**).  
807

## 808 METHODS

809 **General experimental approaches.** No samples, mice, human research participants and data  
810 points were excluded from the reported analysis. Randomization was not performed. Analyses  
811 were not blinded except when noted below. Detailed information and description of common  
812 techniques are described in Supplementary Methods as indicated below.

### 813 **Culture of human adipocytes and in vitro measurements.**

814 Culture of adipocytes. hMADS cells were expanded in DMEM 5.5 mM glucose (Lonza)  
815 supplemented with 10% fetal bovine serum (Lonza), 2 mM L-glutamine (Invitrogen), 10 mM  
816 HEPES buffer (Lonza), 50 units/ml of penicillin (Invitrogen), 50 mg/ml of streptomycin  
817 (Invitrogen), supplemented with 2.5 ng/ml of fibroblast growth factor 2 (Sigma). At  
818 confluence, fibroblast growth factor 2 was removed from proliferation medium. On the next  
819 day (day 0), the cells were incubated in differentiation medium (DMEM/Ham's F-12 medium  
820 containing 7.8 mM glucose, HEPES, L-glutamine, penicillin/streptomycin, 10 µg/ml of  
821 transferrin (Sigma), 10nM of insulin (Sigma), 0,2 nM triiodothyronine (Sigma), 100 µM 3-  
822 isobutyl-1-methylxanthine (Sigma), 1 µM dexamethasone (Sigma), and 100 nM rosiglitazone  
823 (Sigma)). At days 3 and 10, respectively, dexamethasone and 3-isobutyl-1-methylxanthine,  
824 and then rosiglitazone were removed from culture medium. The experiments were carried out  
825 between days 12 and 15.

826 For primary culture and differentiation of human preadipocytes, subcutaneous adipose tissue  
827 samples were obtained from 5 women (age  $39 \pm 9$  years; BMI  $28 \pm 4$  kg/m<sup>2</sup>) undergoing  
828 elective plastic surgery in the abdominal or dorsal region at Rangueil Hospital, Toulouse,  
829 France. Adipose tissue was cleaned from blood vessels and fibrous material, minced into  
830 pieces and digested in 1 volume of collagenase I (300 units/ml, Sigma) for 90 min in 37 °C  
831 shaking water bath. Digested tissue was filtered through 250 µm strainer, diluted with  
832 PBS/gentamycin and centrifuged at 1300 rpm for 5 min. Pellet was incubated in erythrocyte

833 lysis buffer for 10 min at room temperature. Cells were filtered, centrifuged, resuspended in  
834 PM4 medium with 132 nmol/L insulin for differentiation and collected at day 13<sup>51,52</sup>. The  
835 study was approved by the Ethics Committee of Toulouse University Hospitals (Comité de  
836 Protection des Personnes Sud Ouest et Outre Mer 2, DC-2014-2039). The volunteers signed  
837 informed consent for anonymous use of samples.

838 HEK293 and HepG2 cell cultures. See Supplementary Methods.

839 RNA interference. RNA interference was achieved by small interfering RNA (siRNA).  
840 Briefly, on day 7 and day 4 of differentiation respectively, hMADS and primary  
841 preadipocytes were detached from culture dishes with trypsin/EDTA (Invitrogen) and  
842 counted. Control small interfering RNA against Green Fluorescent Protein (siCTR) and gene-  
843 specific siRNA for HSL, ChREBP, ELOVL6 and ATGL (Eurogentec) were delivered into  
844 adipocytes using a microporator (Invitrogen) with the following parameters: 1,100 V, 20 ms,  
845 1 pulse. The targeted sequences are provided in Supplementary Methods.

846 Adenoviral infection. Adenoviruses encoding under the control of a cytomegalovirus  
847 promoter, ELOVL6 (ADV-207862), the short form of HSL, both in tandem with GFP, or GFP  
848 alone (catalog No. 1060) were obtained from Vector Biolabs. Adenoviral particles  
849 (multiplicity of infection, 200) were added in the culture medium for 24 hours at day 11-12 of  
850 hMADS cell differentiation. Medium was changed and experiments were carried out 48 hours  
851 later.

852 Plasmid transfection. See Supplementary Methods.

853 Oleic acid supplementation in human adipocytes. See Supplementary Methods.

854 Treatments with enzyme inhibitors. For FAS, SCD and ELOVL6 inhibition, hMADS  
855 adipocytes were respectively treated with 1 $\mu$ M of compound AZ12756122 (ex 117 from  
856 WO2008075070A1, synthesized at AstraZeneca), 75nM of A939572<sup>24</sup> (Tocris Biosciences)  
857 and 1w<sup>23</sup> (provided by AstraZeneca) in culture medium for 48 hours. To study the effect of

858 bioactive FA on the induction of ChREBP, cells were treated for 8h with 10 $\mu$ M of triacsin C  
859 (Sigma), an inhibitor of acyl-CoA synthase, in the culture medium.  
860 Gene expression analysis. See Supplementary Methods.  
861 Characterization of human ChREBP $\beta$ -specific exon. See Supplementary Methods.  
862 Western blot analysis. See Supplementary Methods.  
863 Metabolic measurements. Triacylglycerol hydrolase activity was measured on cell extracts <sup>14</sup>.  
864 For other metabolic measurements, insulin was removed from culture medium the day before  
865 the assay. To determine glucose uptake, cells were incubated 50 min at 37°C with or without  
866 100 nM insulin. Then, 125  $\mu$ M of cold 2-deoxy-Dglucose and 0.4  $\mu$ Ci 2-deoxy-D-[<sup>3</sup>H]  
867 glucose (Perkin Elmer) per well were added for 10 min incubation. Culture plates were put on  
868 ice and rinsed with 10 mM glucose in ice-cold PBS and then with ice-cold PBS. Cells were  
869 scraped in 0.05N NaOH, and radioactive 2-deoxy-D-glucose uptake was measured by liquid  
870 scintillation counting of cell lysate. To determine glucose oxidation, cells were incubated for  
871 3 h in Krebs Ringer buffer supplemented with 2% BSA, 10 mM HEPES, 2 mM glucose, and  
872 1  $\mu$ Ci D-[<sup>14</sup>C(U)]glucose (PerkinElmer) with or without 100 nM insulin. A 2x2 cm Whatman  
873 3M paper was placed on top of each well and soaked with 120  $\mu$ L NaOH 1N. After  
874 incubation, filter-trapped <sup>14</sup>CO<sub>2</sub> was measured by liquid scintillation counting. Medium was  
875 acidified with 1M sulfuric acid and medium <sup>14</sup>CO<sub>2</sub> was trapped by benzethonium hydroxide,  
876 during 2 h incubation. Benzethonium-trapped <sup>14</sup>CO<sub>2</sub> was measured by liquid scintillation  
877 counting. Specific activity was counted and used to determine the quantity of oxidized  
878 glucose equivalent. To assess glucose incorporation into FA, cells were then washed twice in  
879 PBS and then scraped in STED. Neutral lipids were extracted in methanol/chloroform (1:2).  
880 Organic phase was dried under nitrogen and hydrolyzed in 1mL 0.25N NaOH in  
881 methanol/chloroform (1:1) for 1 h at 37°C. The solution was neutralized with 500  $\mu$ L 0.5N  
882 HCl in methanol. FAs and glycerol were separated by adding 1.7 mL chloroform, 860  $\mu$ L



883 water, and 1 mL methanol/chloroform (1:2). Incorporation of  $^{14}\text{C}$  into FAs was measured by  
884 liquid scintillation counting of the lower phase. Specific activity was counted and used to  
885 determine the quantity of incorporated glucose equivalent. *De novo* lipogenesis was also  
886 measured using acetic acid-sodium salt-[1- $^{14}\text{C}$ ] (PerkinElmer). Cells were incubated for 3h in  
887 Krebs buffer supplemented with 10mM HEPES, 2mM glucose, 2%BSA and 2 $\mu\text{Ci/mL}$  of  
888 radiolabelled acetate stimulated with or without 100nM insulin. Cells were then washed twice  
889 and harvest in PBS/SDS 0.1%. Neutral lipids were extracted in methanol/chloroform (1:2)  
890 method. Incorporation of  $^{14}\text{C}$  into neutral lipids was measured by liquid scintillation counting  
891 of lower phase. Results from metabolic measurements were normalized to total protein  
892 content of cell extracts.

893 ELOVL6 activity. Fatty acid elongation activity was measured in crude microsomal extracts  
894 from hMADS adipocytes<sup>41</sup>. Briefly, cells were washed with PBS, scraped in 3 ml of ice-cold  
895 250m M sucrose, Hepes 20 mM, EDTA 1 mM, pH 7.5 and dounce-homogenized.  
896 Homogenate was centrifuged 1000 g at 4°C for 7 min. Supernatant was collected and  
897 centrifuged at 2000 g at 4°C for 30 min. Supernatant was collected and centrifuged at 17000 g  
898 at 4°C for 1 hour. The resultant pellet was suspended in 50  $\mu\text{l}$  of 100 mM Tris-HCl, pH 7.4  
899 and used for fatty acid elongation activity after determination of protein concentration.  
900 ELOVL6 activity was assayed by the measurement of [2- $^{14}\text{C}$ ]malonyl-CoA (Perkin Elmer)  
901 incorporation into exogenous palmitoyl-CoA<sup>53</sup>. ELOVL6 inhibitor (1 $\mu\text{M}$  of compound 1w<sup>23</sup>)  
902 was preincubated 30 min at 37°C with microsomal protein before addition of reaction  
903 mixture. Incubation was stopped by adding 0.2 ml of 5M KOH, 10% methanol and saponified  
904 at 65°C for 1h. Then the samples were cooled and acidified with 0.2 ml of ice-cold 5N HCl  
905 and 0.2 ml of ethanol. Free fatty acids were extracted from the mixture three times with 1 ml  
906 of hexane, 2% acetic acid. The pooled hexane fractions were dried under nitrogen and after  
907 addition of 3 ml of scintillation cocktail, the radioactivity incorporated was counted. Blanks

908 were carried out in parallel reactions incubated without microsomal fractions. ELOVL6  
909 activity obtained by subtracting [<sup>14</sup>C]malonyl-CoA molecules incorporated into fatty acids in  
910 the absence of inhibitor to the values in the presence of the ELOVL6 inhibitor.

911 Chromatin immunoprecipitation assays. Human adipocyte cells (10<sup>7</sup> cells per condition) were  
912 fixed with 1% formaldehyde for 15 min and quenched with 0.125 M glycine. Chromatin was  
913 isolated by the addition of lysis buffer, followed by disruption with a Dounce homogenizer.  
914 Lysates were sonicated and the DNA sheared to an average length of 300-500 bp. Genomic  
915 DNA (Input) was prepared by treating aliquots of chromatin with RNase, proteinase K and  
916 heat for de-crosslinking, followed by ethanol precipitation (Active Motif Inc.). Pellets were  
917 resuspended and the resulting DNA was quantified on a NanoDrop spectrophotometer.  
918 Extrapolation to the original chromatin volume allowed quantitation of the total chromatin  
919 yield. Aliquots of chromatin (30 µg) were precleared with protein A agarose beads  
920 (Invitrogen). Genomic DNA regions of interest were isolated using an antibody against  
921 ChREBP (Novus, cat# NB400-135). Positive and negative controls were designed by Active  
922 Motif Inc. Complexes were washed, eluted from the beads with SDS buffer, and subjected to  
923 RNase and proteinase K treatment. Crosslinks were reversed by incubation overnight at 65 C,  
924 and ChIP DNA was purified by phenol-chloroform extraction and ethanol precipitation.  
925 Quantitative PCR reactions were carried out in triplicate using SYBR Green Supermix (Bio-  
926 Rad, Cat # 170-8882) on a CFX Connect™ Real Time PCR system. Positive and negative  
927 control sites were tested for each factor as well as the sites of interest. The resulting signals  
928 were normalized for primer efficiency by carrying out qPCR for each primer pair using input  
929 DNA (pooled unprecipitated genomic DNA from each sample).

930 Cellular subfractionation. Nuclear and cytosolic fractions from hMADS adipocytes were  
931 prepared using Nuclear Extract Kit (40010) from Active Motif. Cells were rinsed with PBS  
932 and immediately scrapped into 1X Hypotonic Buffer. For adipose tissue, tissues were ground

933 in liquid nitrogen and lysed using the NE-PER nuclear and cytoplasmic extraction reagent kit  
934 (Thermoscientific, 78835). Subsequent steps followed the manufacturer's protocol. Anti-  
935 histone H3 (4499, Cell Signaling Technology), anti-lamin A/C (4777, Cell Signaling  
936 Technology) and  $\alpha$  tubulin (T5168, Sigma) antibodies were used to analyze the efficiency of  
937 cellular fractionation.

938 Fatty acid composition of TG and PL. Cells were scraped in PBS and then mixed with  
939 methanol supplemented with butylated hydroxytoluene (BHT) 0.001%. Lipid extraction was  
940 performed with a chloroform/methanol mixture (1:1) and KCl (0.5 M) after centrifugation  
941 (2500rpm, 10min). PL and TG were isolated by thin-layer chromatography on silica glass  
942 plates (Merck) using petroleum ether/diethyl ether/acetic acid (80:20:1) as the mobile phase.  
943 FA methyl esters were generated by transmethylation of the glycerolipids in methanol with 5  
944 % acetyl chloride at 60°C for 1 hour, extracted 2 times by isooctane. Analysis was carried out  
945 with a gas chromatograph (Shimadzu GC 2100) equipped with a CP-Wax 58 capillary  
946 column, 50 m in length, 0.25-mm external diameter and 0.2- $\mu$ m thickness of the stationary  
947 phase (Varian Inc.), with helium 1 mL/min as carrier gas. Programmed temperature  
948 vaporization (PTV system) injector and flame ionization detector were used. Results are  
949 expressed in percentage of total FA contained in the sample.

950 Fatty acid composition in phospholipid classes. See Supplementary Methods.

951 Measurement of glycerol and NEFA in culture medium. See Supplementary Methods.

952 Fluorescence recovery after photobleaching (FRAP). Cells were labeled for 15 min with  
953 1 $\mu$ g/ml Alexa 555-labeled Cholera ToxinSubunit B (CTxB Molecular Probes) at 4°C, then  
954 washed three times in chilled medium supplemented with 25 mM HEPES buffer, pH 7.4.  
955 LSM780 confocal microscope, equipped with a high sensitive 32 channel GaAsP detectors,  
956 operated with Zen Black software, coupled to a DPSS-laser (561 nm, maximum power 20  
957 mW) was used for excitation with a detection bandwidth of 571-624 nm (Carl Zeiss). All

958 experiments were done at room temperature (22°C). Cells were observed using a Plan-  
959 Apochromat 63X NA 1.4 oil immersion objective, and the pixel dwell was set to the optimal  
960 value of 1.92 μs. The fluorescence intensity of three regions of interest of 6.4 μm×3.2 μm was  
961 measured: the photobleached area, a region within the cell that was not photobleached to  
962 check for overall photobleaching and cell position fluctuation and the background. After 10  
963 prebleach scans (one scan every 200 ms) at 1% maximal laser power to determine initial  
964 fluorescence intensity, one photobleaching scan was performed at 100% laser power. Post  
965 bleach fluorescence recovery was then sampled at 1% laser power for 150 s. FRAP data  
966 analysis was done using the method described by Bonneau *et al.*<sup>54</sup>.

967 Immunoprecipitation. HEK293T cells were harvested in a lysis buffer containing 3% NaCl  
968 5M, 5% TrisHCl 1M (pH 7.5), 1% EDTA 500mM, 1.338% PPiNa and 0.02% NaF,  
969 supplemented with 1% Triton X-100 (Sigma), 2% 50X protease inhibitor cocktail (Roche)  
970 and 1% 1mM orthovanadate (Sigma). 1 mg of proteins was immunoprecipitated overnight at  
971 4°C, with 40μL of anti-FLAG M2 magnetic beads (Sigma). Beads were gently centrifuged for  
972 1 min and washed with the lysis buffer before elution in Laemmli buffer.

973 For immunoprecipitation between purified proteins, FLAG-tagged ChREBP was expressed in  
974 HEK293T cells. Cells were harvested in lysis buffer described above. 300 μg of proteins was  
975 immunoprecipitated overnight at 4°C with 40μL of anti-FLAG M2 magnetic beads. Beads  
976 bound with ChREBP were washed with the lysis buffer and incubated with 1 μg of human  
977 recombinant HSL (Cayman) in 350 μl of lysis buffer for 3h at 4 °C with gentle rocking. The  
978 beads were washed 3 times with lysis buffer.

979 For endogenous co-immunoprecipitation in hMADS adipocytes, cells were lysed for 15min in  
980 1X hypotonic buffer (Active Motif) with 4% 25X protease inhibitor cocktail (Roche) and  
981 1mM orthovanadate (Sigma). Cell debris and fat was discarded after 12700rpm centrifugation  
982 at 4°C for 15min. Preclearing was performed at 4°C for 30min using 50μL protein G and 4μg

983 control rabbit (2729, Cell Signaling Technology) or mouse (sc-2025, Santa Cruz) IgG. Beads  
984 were discarded and supernatants were incubated with 4µg anti-ChREBP (NB400-135, Novus)  
985 ou 2µg anti-HSL (sc-74489, Santa Cruz Biotechnology) for 90 min at 4°C. As negative  
986 control of immunoprecipitation, 4µg control rabbit (2729, Cell Signaling Technology) or 2µg  
987 mouse (sc-2025, Santa Cruz) IgG were used. Protein A/protein G (50:50) magnetic beads  
988 were added for 1h at 4°C. Beads were washed in cold PBS with 4% 25X EDTA and 1mM  
989 orthovanadate.

990 For ChREBP immunoprecipitation in mouse white adipose tissue, fat was cut in small pieces  
991 and lysed during 2h in 20 mM Tris/HCl, 150 mM NaCl, 0.5% NP-40 and  
992 protease/phosphatase inhibitors; pH 8. Following centrifugation at 15000g for 20 min at 4°C,  
993 fat layer was removed before collecting the supernatant. For each immunoprecipitation, 0.8 to  
994 1 mg of protein was precleared with 50 µl of Protein A Dynabeads (ThermoFisher) for 1h at  
995 4°C, then incubated overnight at 4°C with 40µl Protein A dynabeads coupled with 5µg Rabbit  
996 IgG or ChREBP antibody (Novus). Beads were washed 4 time with lysis buffer prior elution  
997 in 2X Laemmli buffer.

998 In situ proximity ligation assay and immunofluorescence. In situ proximity ligation assay was  
999 performed using Duolink In Situ reagents (Sigma). Cells and pieces of subcutaneous adipose  
1000 tissue were fixed with 4% paraformaldehyde (Sigma) and permeabilized 15 min at room  
1001 temperature with 0.2% Triton X-100 (Sigma). Incubation of antibodies, ligation of  
1002 oligodeoxynucleotides and amplification were performed following manufacturer's  
1003 instructions. The following primary antibodies were incubated overnight at 4°C: anti-HSL  
1004 (murine antibody, sc-74489, Santa Cruz Biotechnology), anti-ATGL (mouse antibody, NBP2-  
1005 59390, Novus), anti-AKT (mouse antibody, 2920, Cell Signaling Technology) and anti-  
1006 ChREBP (rabbit antibody, NB400-135, Novus). The same antibodies were used in  
1007 immunofluorescence assays. Anti-mouse (Alexa-fluor 488-conjugated, A21202, and Alexa-

1008 fluor 546-conjugated, A10036, Invitrogen) and anti-rabbit (Alexa-fluor 546-conjugated,  
1009 Invitrogen) secondary antibodies were incubated at 1/300 for 45min. Neutral lipids were  
1010 stained using Bodipy (4-3922, Life Technologies) for 30min. Nucleus labeling was performed  
1011 using Hoescht (33342, 5mg/mL, Invitrogen) for 5 min. Confocal microscopy was performed  
1012 using Zeiss LSM780. Image processing was similar for all conditions. The same settings were  
1013 applied to entire images.

1014 Surface Plasmon Resonance assays. All binding studies based on surface plasmon resonance  
1015 technology were performed on BIAcore T200 optical biosensor instrument (GE Healthcare).  
1016 Immobilization of anti-ChREBP antibody (NB400-135, Novus) was performed by the Fc  
1017 region to the chip surface using native Protein A sensorchip in PBS-P+ buffer (20mM  
1018 Phosphate Buffer pH 7.4, 2.7mM Kcl, 137mM NaCl, and 0.05% surfactant P20) (GE  
1019 Healthcare). Immobilization step were performed at a flow rate of 5 µl/min with a final  
1020 concentration of 2µg/ml. Total amount of immobilized antibody was 11000-12000RU. Then  
1021 all injection steps were performed at a flow rate of 20µl/min. Channel Fc1 was used as a  
1022 reference surface for non-specific binding measurements.

1023 Luciferase activity. See Supplementary Methods.

1024

1025 **Animal studies.** No randomization and blinding was performed. Animals from several litters  
1026 were used in each protocol to avoid litter-to-litter variation.

1027 Mouse models. Targeted disruption of the *Lipe* gene and generation of *Lipe*<sup>+/-</sup> mice have been  
1028 described elsewhere<sup>13</sup>. Before euthanasia, mice were fasted for 24h or refed for 18h  
1029 supplemented with 20% glucose in drinking water. To create transgenic mice with specific  
1030 deletion of *Lipe* exon B, mRNA coding for zinc finger nucleases targeting specifically HSL  
1031 (CompoZr™ Custom Zinc Finger Nucleases, CSTZFN-1KT, Sigma) was injected into  
1032 pronuclei of one-cell embryos from female B6D2/F1 mice. Homozygous mice (*Lipe*<sup>exonB-/-</sup>)

1033 registered as B6D2-*Lipe*<sup>em1L<sup>and</sup></sup> mice) were obtained. Full description of the model will be  
1034 published elsewhere. The specific inhibitor of HSL (BAY 59-9435) was synthesized by  
1035 NoValix (Illkirch, France)<sup>55</sup>. Transgenic mice were fed high fat diet (60% or 45% kcal fat,  
1036 respectively, D12492 and D12451 from Research Diets) for indicated times. In  
1037 pharmacological studies, C57BL/6J male mice (12-15 weeks-old, Janvier Laboratories) were  
1038 treated orally with DMSO or HSL inhibitor (70mg/kg once daily) for 11 days. Eight week-old  
1039 DBA2/J and C57BL6/J male mice (Charles River) were fed high fat diet (60% kcal fat,  
1040 D12492 from Research Diets) for 6 weeks before sacrifice. Mice were housed and  
1041 manipulated according to Inserm guidelines and European Directive 2010/63/UE in the local  
1042 animal care facility (agreements A 31 555 04 and C 31 555 07). Protocols were approved by  
1043 the French Ministry of Research following review by local ethical committee (CEEA122).  
1044 In studies on ChREBP null mice, 10 to 12 week-old male and female *Mlxipl* global knockout  
1045 mice<sup>56</sup> and wild-type littermates were maintained in a 12-h light/dark cycle with water and  
1046 chow diet (65% carbohydrate, 11% fat, and 24% protein). For fasting-refeeding experiment,  
1047 mice were either fasted for 24h (fasted group) or refed for 18h on chow diet and had access to  
1048 drinking water with 20% glucose, following a 24h fast (Refed group). Mice were housed and  
1049 manipulated according to Inserm guidelines and European Directive 2010/63/UE in the local  
1050 animal care facility (agreement A751320). Protocols were approved by the French Ministry of  
1051 Research following review by local ethical committee (CEEA34).  
1052 Mice homozygous for a deletion in *Elovl6* and their wild-type littermates were phenotyped on  
1053 a C56BL6/J background<sup>57</sup>. The research has been regulated under the Animals (Scientific  
1054 Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the  
1055 University of Cambridge Animal Welfare and Ethical Review Body (AWERB).  
1056 Gene and protein expression analyses. See Supplementary Methods.  
1057 Measurement of fasting glucose and insulin. See Supplementary Methods.

1058 Glucose and insulin tolerance tests and insulin bolus injection. See Supplementary Methods.

1059 Euglycemic-hyperinsulinemic clamp. See Supplementary Methods.

1060 **Human research.** Nature of the groups was blinded to the investigator performing gene  
1061 expression experiments.

1062 Women with differing obese and metabolic status. Participating women (lean group mean age  
1063  $37 \pm 16$  years; obese with metabolic syndrome group mean age,  $49 \pm 11$  years) were  
1064 scheduled to have abdominal surgery (laparoscopic or laparotomic cholecystectomy and  
1065 gastric banding) <sup>58</sup>. During the surgical procedure, samples of visceral adipose tissue were  
1066 obtained by surgical excision. Euglycemic hyperinsulinemic clamp was performed at rest  
1067 after an overnight fast. Each subject gave written informed consent and the study was  
1068 approved by the Ethics Committee of the Third Faculty of Medicine, Charles University,  
1069 Prague.

1070 Hyperglycemic hyperinsulinemic clamp. The 8 participating men were  $23 \pm 3$  years-old  
1071 (BMI,  $23 \pm 2$  kg/m<sup>2</sup>). The hyperglycemic hyperinsulinemic clamp was a modification of the  
1072 hyperglycemic method used by Del Prato et al. combined with the original hyperinsulinemic  
1073 clamp described by DeFronzo <sup>59,60</sup>. For hyperglycemia, the objective was to increase plasma  
1074 glucose 5.5 mmol/l above fasting level by infusing 20% dextrose in two phases: 1) bolus dose  
1075 to increase glycemia to the desired target and 2) continuous infusion dose adjusted every 5–10  
1076 min according to measured plasma glucose to maintain glycemia at the desired target. To  
1077 obtain hyperinsulinemia, insulin was co-infused at the rate of  $75$  mU/m<sup>2</sup>·min for 180 min. The  
1078 study was approved by the Ethics Committee of University of Montreal. The volunteers gave  
1079 their written consent after being informed of the nature, purpose, and possible risks of the  
1080 study.

1081 Morbidly obese subjects undergoing bariatric surgery. This cohort has in part been described  
1082 before <sup>61</sup>. In brief, 14 obese women (BMI > 35 kg/m<sup>2</sup>; age,  $48 \pm 9$  years) referred to the



1083 hospital for gastric by-pass surgery (Roux-en-Y) were investigated before surgery and 2 years  
1084 post-operatively. According to self-report, body weight had been stable ( $\pm 2$  kg) for at least 3  
1085 months prior to both investigations. The study was approved by the regional ethics board in  
1086 Stockholm and registered at clinicaltrials.gov as NCT01785134. Subjects were randomized to  
1087 omentectomy or not and this was blinded to investigators and patients. Procedure was  
1088 explained in detail to each women and written informed consent was obtained.

1089 Gene expression analysis. See Supplementary Methods.

1090

1091 **Statistical analysis.** Results from biological replicates were expressed as mean  $\pm$  SEM.  
1092 Statistical analyses were performed using GraphPad Prism (GraphPad Software v.5.0).  
1093 D'Agostino and Pearson omnibus normality test was used to test normality. Fischer test was  
1094 used to test for equality of variances. Data were Log transformed when appropriate to reach  
1095 normality and uniform distribution. Statistical tests were two-sided. Paired or unpaired  
1096 Student's t tests, Wilcoxon's test and, Mann and Whitney's test were performed to compare  
1097 two conditions. Paired or unpaired one-way ANOVA and Friedman's tests were performed  
1098 and followed respectively by Bonferroni's and Dunn's post hoc tests to determine differences  
1099 between several groups. Paired or unpaired two-way ANOVA with Bonferroni's post hoc  
1100 tests were used to compare two variables. Linear regression was used to test association  
1101 between two variables.

1102

1103 **Data availability statement.** The data that support the plots within this paper and other  
1104 findings of this study are available from the corresponding author upon reasonable request.

1105

1106 METHODS-ONLY REFERENCES

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