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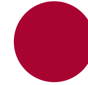

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Interaction between Hormone-Sensitive Lipase and ChREBP in Fat Cells Controls Insulin Sensitivity

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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 ChREBPα, independently of lipase catalytic activity, impairs ChREBPα translocation into the
72 nucleus and induction of ChREBPβ, 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 [1]. Direct manipulation of
85 the fat cell insulin signaling pathway in mice also supports the systemic importance of
86 adipose tissue [2-4]. Defects in adipose insulin signaling have been reported in insulin resistant
87 and type 2 diabetic patients [5-8]. 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 [9,10]. 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 [11]. 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 [12]. 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 [13].

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 [14,15], 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 [16,17], 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 [13]. 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 [18]. $Lipe^{exonB-/-}$ 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**). $Lipe^{exonB-/-}$ 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 [19-22]. To investigate the respective contribution of the two steps, specific
237 inhibitors were used. Treatment of human adipocytes with an inhibitor of ELOVL6 [23] 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 [24] 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 [19,25,26]. 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 [27]. Adipose ChREBP is a major
268 determinant of systemic insulin action on glucose metabolism [28]. 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. ChREBPα, which transcriptional activity is
290 regulated by glucose, and ChREBPβ, a transcriptionally superactive and unstable isoform
291 which is a direct transcriptional target of ChREBPα [28,29]. We characterized the human β-
292 specific exon of *MLXIPL* which extends 29 deoxynucleotides 3' of its mouse counterpart [28]
293 (**Supplementary Fig. 5c**). In human adipocytes with siRNA-mediated knock down of HSL,
294 the levels of *ChREBP* transcripts, notably the β 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 [27]. 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 [14]. 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 ChREBPα with FLAG epitope tag [30]. Immunoprecipitation of cell lysates with anti-FLAG
328 IgG and immunoblotting with anti-HSL antibody showed that HSL co-immunoprecipitated
329 with ChREBPα (**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 ChREBPα 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 ChREBPα further indicating that HSL interaction with
336 ChREBPα is independent lipolysis and specific to this neutral lipase (**Fig. 6c**).
337 Furthermore, interaction of HSL with ChREBPα 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 [31]. 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 ChREBPα 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 ChREBPα 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 ChREBPα 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 ChREBPα 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 ChREBPα
360 protein content in fat pads of the two genotypes (**Supplementary Fig. 7d**). To evaluate the
361 effect of HSL on ChREBPα 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 [32]. Promoter activity increased when cells expressed ChREBPα and
364 decreased when cells co-expressed increasing amounts of HSL (**Fig. 6i, Supplementary Fig.**
365 **7e**). The data suggest that HSL binds to ChREBPα and sequesters the transcription factor in
366 the cytoplasm. Upon decrease of HSL expression, HSL-ChREBP interaction is diminished,
367 ChREBPα 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**) [33,34]. 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 ChREBPα protein in adipocytes
383 expressing HSL-S and in the absence of correlation between HSL and ChREBPα 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 [35]. Partial inhibition of the fat cell neutral lipase,
392 HSL, alleviates insulin resistance without increasing body weight, two essential requirements
393 for therapeutic interventions [13] (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 [36,37]. *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 β isoform, has been reported [16,28,38]. 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 ChREBPβ 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 [16,39]. Of note, in monozygotic
409 twin pairs discordant for type 2 diabetes, adipose *ELOVL6* is markedly lower in the affected
410 twins [40]. 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 [22,41]. 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 [42,43]. This effect may contribute to the decreased incidence of type 2 diabetes in patients at risk
417 fed Mediterranean diets [43]. At the cellular level, monounsaturated fatty acids have been
418 reported to protect from the damaging effect of palmitic acid on insulin signaling [19,26]. 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 [25,44,45]. 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 [41,46].
427 The role of HSL in human fat cell lipolysis is well established [14,47]. 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 [48]. 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 [29]. However, although we cannot rule out that, in some
433 conditions, upregulation of ChREBPα 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 ChREBPα 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 [33]. 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 [29,49]. 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 [50]. 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
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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 ±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*$^{+/-}$, 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*$^{+/-}$ 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*$^{exonB-/-}$, 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*$^{exonB-/-}$ 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*$^{exonB-/-}$ 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*$^{exonB-/-}$ 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*-/-, 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 ±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, $^{\$\$}$P<0.01,
693 $^{\$\$\$}$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), 1µM 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), 100µM (O100) or 500µM (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, 50µm. (**l**) Calculated mobile fraction (white bar, n=17 analyzed cells; avocado bar, n=16
722 analyzed cells). Data are mean ±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 $^{$$}$P<0.01, $^{$$$}$P<0.001 compared to HSL-deprived adipocytes. $^{\#}$P<0.05, $^{\#\#}$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*-/-, 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 *ChREBPα* and *ChREBPβ* (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. $^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001 compared to HSL-deprived
761 adipocytes. $^{\#}$P<0.05, $^{\#\#}$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*$^{+/-}$ (+/-) 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, 50µm. (**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, 50µm. (**g**) ChREBPα protein levels in nuclear extracts from control (siCTR) and
783 HSL-deprived (siHSL) hMADS adipocytes (n=8 biologically independent samples per
784 group). (**h**) ChREBPα protein levels in nuclear extracts from white adipose tissue of wild type
785 (WT, n=8 animals) and *Lipe*$^{+/-}$ (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 ChREBPα immunocomplexes in
792 HEK-293 cells transfected with empty plasmid (pcDNA3), FLAG-ChREBP, full lengh HSL
793 (HSL) or short form HSL (HSL-S) expression plasmids. Anti-FLAG antibody was used for
794 immunoprecipitation. β-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

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

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 ± 9 years; BMI 28 ± 4 kg/m^2) 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 [51,52]. 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 1µM of compound AZ12756122 (ex 117 from
856 WO2008075070A1, synthesized at AstraZeneca), 75nM of A939572 [24] (Tocris Biosciences)
857 and 1w [23] (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 10µM 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 ChREBPβ-specific exon. See Supplementary Methods.
862 Western blot analysis. See Supplementary Methods.
863 Metabolic measurements. Triacylglycerol hydrolase activity was measured on cell extracts [14].
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 µM of cold 2-deoxy-Dglucose and 0.4 µCi 2-deoxy-D-[^{3}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 µCi D-[^{14}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 µL NaOH 1N. After
874 incubation, filter-trapped $^{14}CO_2$ was measured by liquid scintillation counting. Medium was
875 acidified with 1M sulfuric acid and medium $^{14}CO_2$ was trapped by benzethonium hydroxide,
876 during 2 h incubation. Benzethonium-trapped $^{14}CO_2$ 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 µL 0.5N
882 HCl in methanol. FAs and glycerol were separated by adding 1.7 mL chloroform, 860 µL

883 water, and 1 mL methanol/chloroform (1:2). Incorporation of ^{14}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}C] (PerkinElmer). Cells were incubated for 3h in
887 Krebs buffer supplemented with 10mM HEPES, 2mM glucose, 2%BSA and 2µCi/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}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 [41]. 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 µl 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 essayed by the measurement of [2-^{14}C]malonyl-CoA (Perkin Elmer)
901 incorporation into exogenous palmitoyl-CoA [53]. ELOVL6 inhibitor (1µM of compound 1w [23])
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 [^{14}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 (10^7 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 α 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-µm 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 1µg/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.* [54].

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*$^{+/-}$ mice have been
1028 described elsewhere [13]. 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*$^{exonB-/-}$

1033 registered as B6D2-*Lipe*^{em1Land} 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) [55]. 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 [56] 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 [57]. 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 ± 16 years; obese with metabolic syndrome group mean age, 49 ± 11 years) were
1064 scheduled to have abdominal surgery (laparoscopic or laparotomic cholecystectomy and
1065 gastric banding) [58]. 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 ± 3 years-old
1071 (BMI, 23 ± 2 kg/m^2). 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 [59,60]. 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^2·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 [61]. In brief, 14 obese women (BMI>35 kg/m^2; age, 48 ± 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 (± 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 ± 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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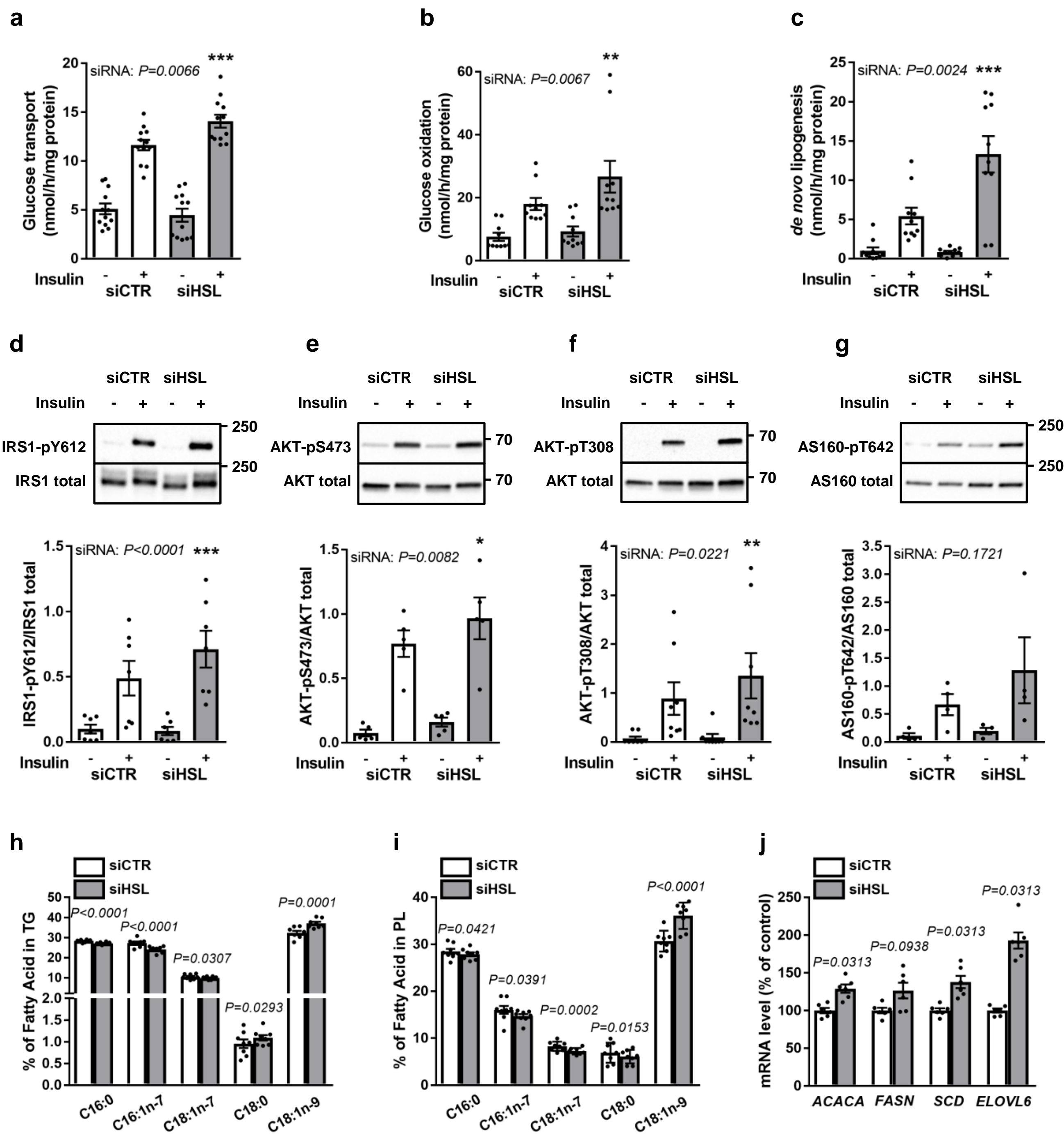
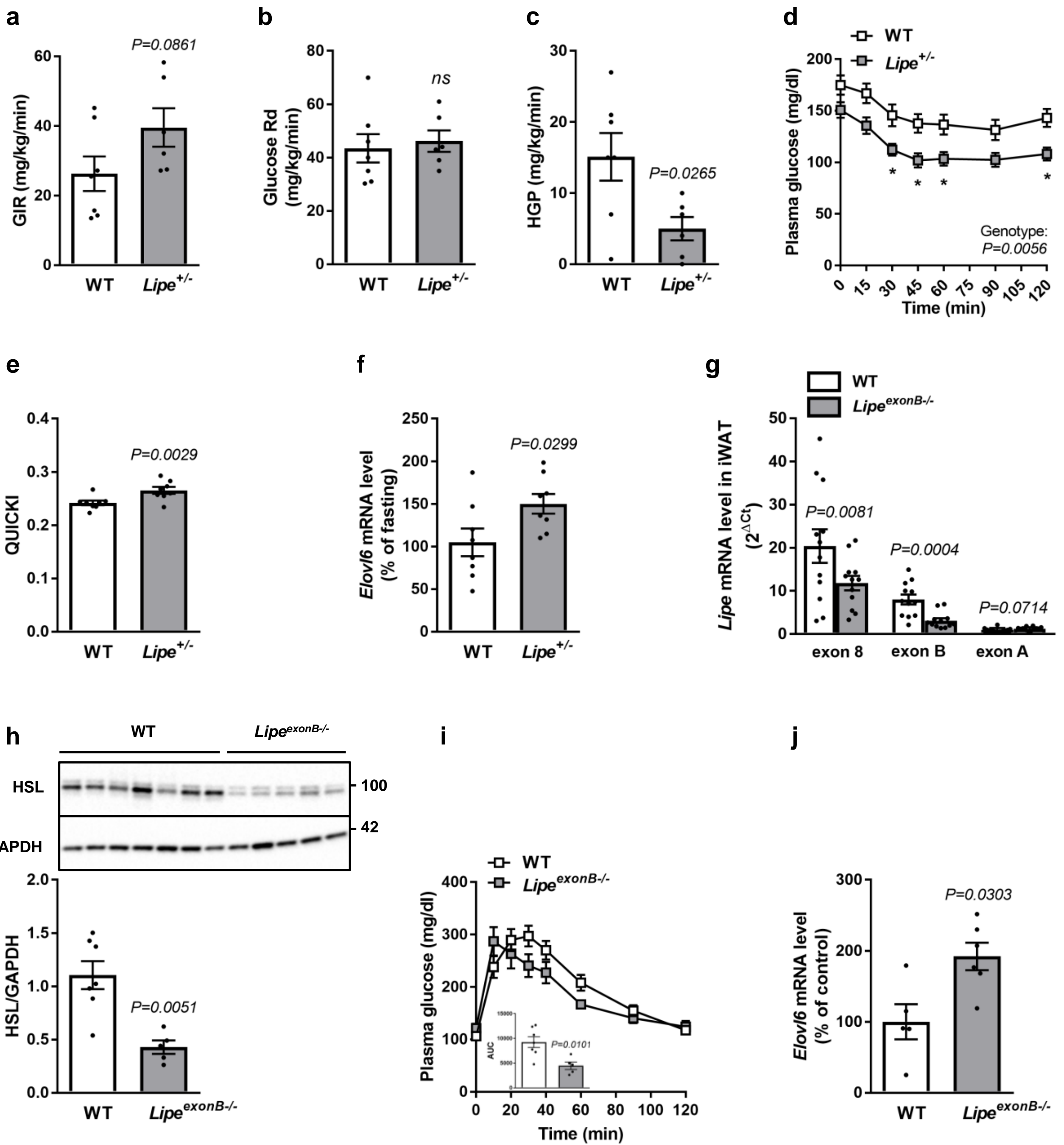
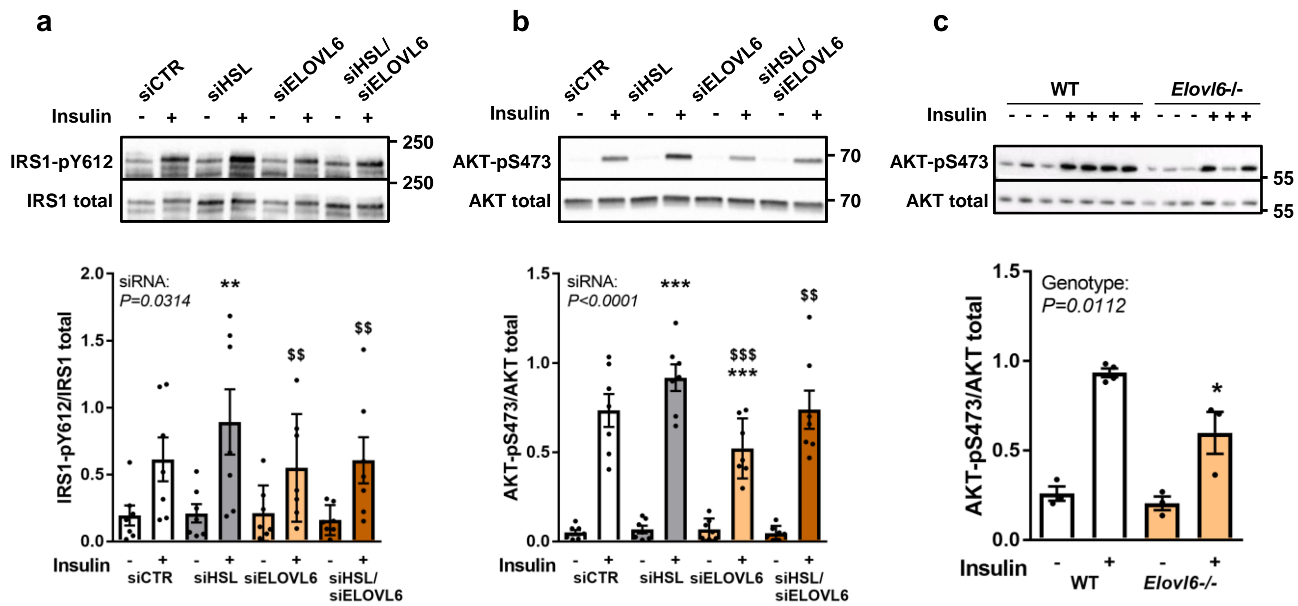
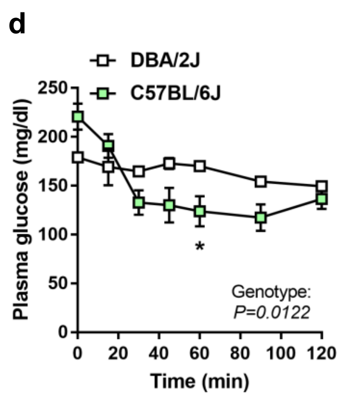


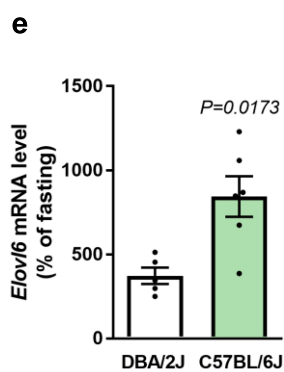
a
siRNA: P=0.0066
***
Glucose transport (nmol/h/mg protein)
Insulin
siCTR siHSL
b
siRNA: P=0.0067
**
Glucose oxidation (nmol/h/mg protein)
c
siRNA: P=0.0024
***
de novo lipogenesis (nmol/h/mg protein)
d
IRS1-pY612
IRS1 total
siRNA: P<0.0001
IRS1-pY612/IRS1 total
e
AKT-pS473
AKT total
siRNA: P=0.0082
AKT-pS473/AKT total
f
AKT-pT308
AKT total
siRNA: P=0.0221
AKT-pT308/AKT total
g
AS160-pT642
AS160 total
siRNA: P=0.1721
AS160-pT642/AS160 total
h
% of Fatty Acid in TG
P<0.0001
P<0.0001
P=0.0307
P=0.0293
P=0.0001
C16:0 C16:1n-7 C18:1n-7 C18:0 C18:1n-9
i
% of Fatty Acid in PL
P=0.0421
P=0.0391
P=0.0002
P=0.0153
P<0.0001
j
mRNA level (% of control)
P=0.0313
P=0.0938
P=0.0313
P=0.0313
ACACA FASN SCD ELOVL6

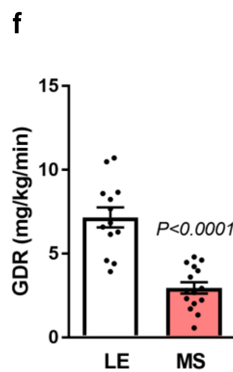


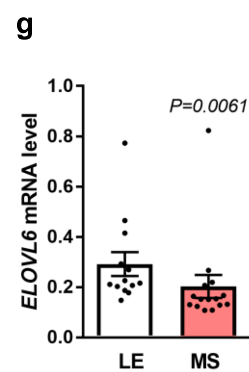
a
GIR (mg/kg/min)
P=0.0861
WT
Lipe+/-
b
Glucose Rd (mg/kg/min)
ns
WT
Lipe+/-
c
HGP (mg/kg/min)
P=0.0265
WT
Lipe+/-
d
WT
Lipe+/-
Plasma glucose (mg/dl)
Genotype:
P=0.0056
Time (min)
e
QUICKI
P=0.0029
WT
Lipe+/-
f
Elovl6 mRNA level (% of fasting)
P=0.0299
WT
Lipe+/-
g
WT
LipeexonB-/-
Lipe mRNA level in iWAT (2ΔCt)
P=0.0081
P=0.0004
P=0.0714
exon 8
exon B
exon A
h
WT
LipeexonB-/-
HSL
GAPDH
100
42
HSL/GAPDH
P=0.0051
WT
LipeexonB-/-
i
WT
LipeexonB-/-
Plasma glucose (mg/dl)
AUC
P=0.0101
Time (min)
j
Elovl6 mRNA level (% of control)
P=0.0303
WT
LipeexonB-/-

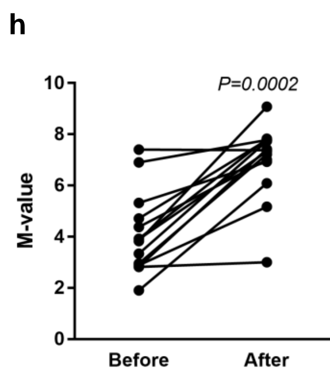

a
siCTR
siHSL
siELOVL6
siHSL/
siELOVL6
Insulin
IRS1-pY612
IRS1 total
250
250
siRNA:
P=0.0314
IRS1-pY612/IRS1 total
siCTR
siHSL
siELOVL6
siHSL/
siELOVL6
b
AKT-pS473
AKT total
70
70
siRNA:
P<0.0001
AKT-pS473/AKT total
c
WT
Elovl6-/-
AKT-pS473
AKT total
55
55
Genotype:
P=0.0112
AKT-pS473/AKT total
Insulin
WT
Elovl6-/-

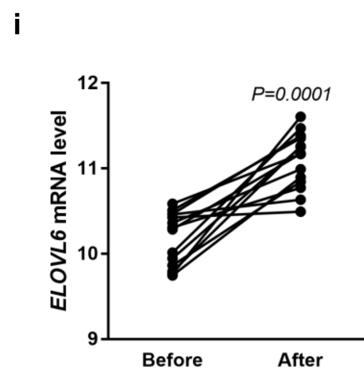

d
DBA/2J
C57BL/6J
Plasma glucose (mg/dl)
Time (min)
Genotype:
P=0.0122

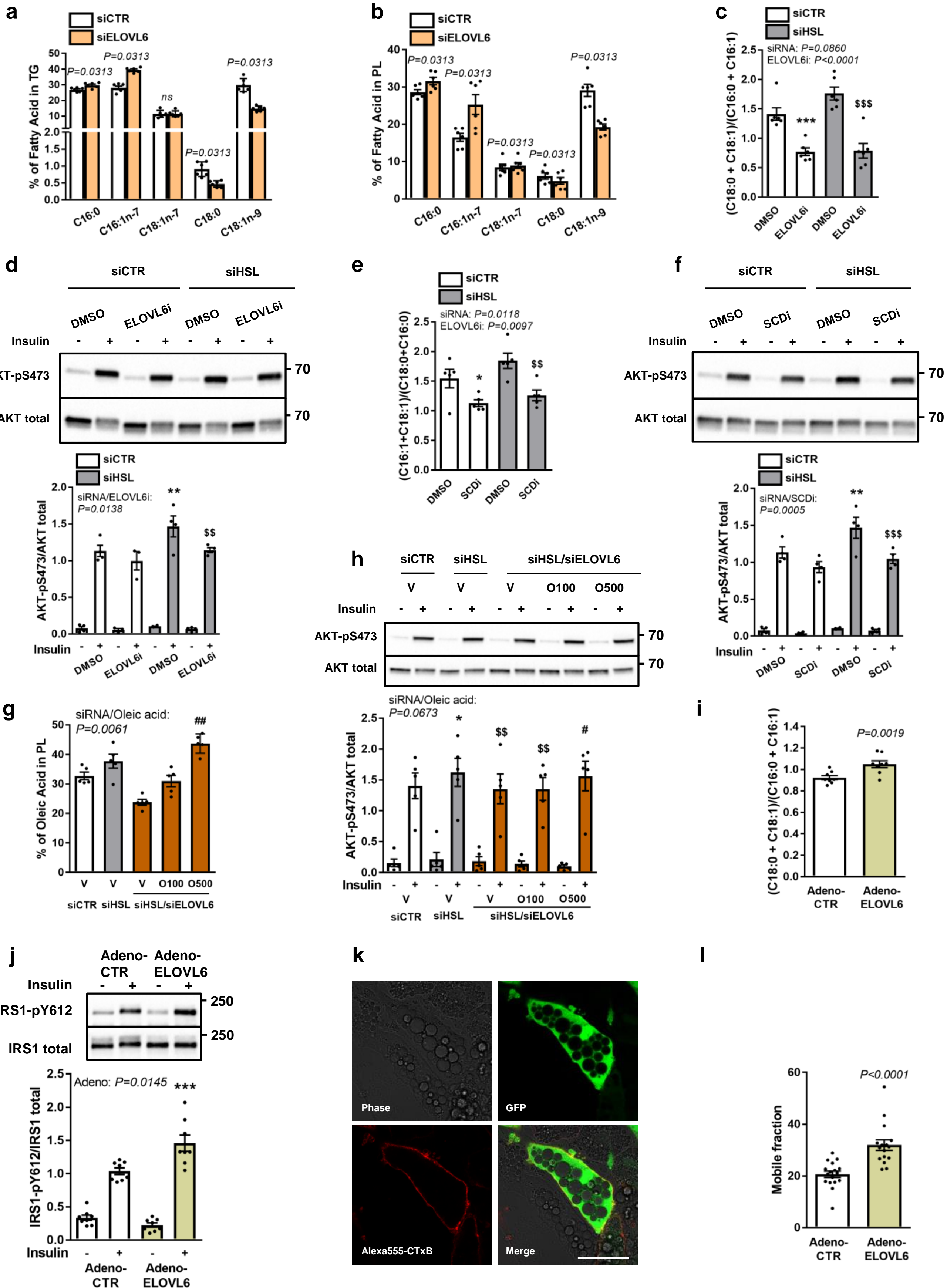

e
Elovl6 mRNA level
(% of fasting)
P=0.0173
DBA/2J
C57BL/6J


f
GDR (mg/kg/min)
P<0.0001
LE
MS

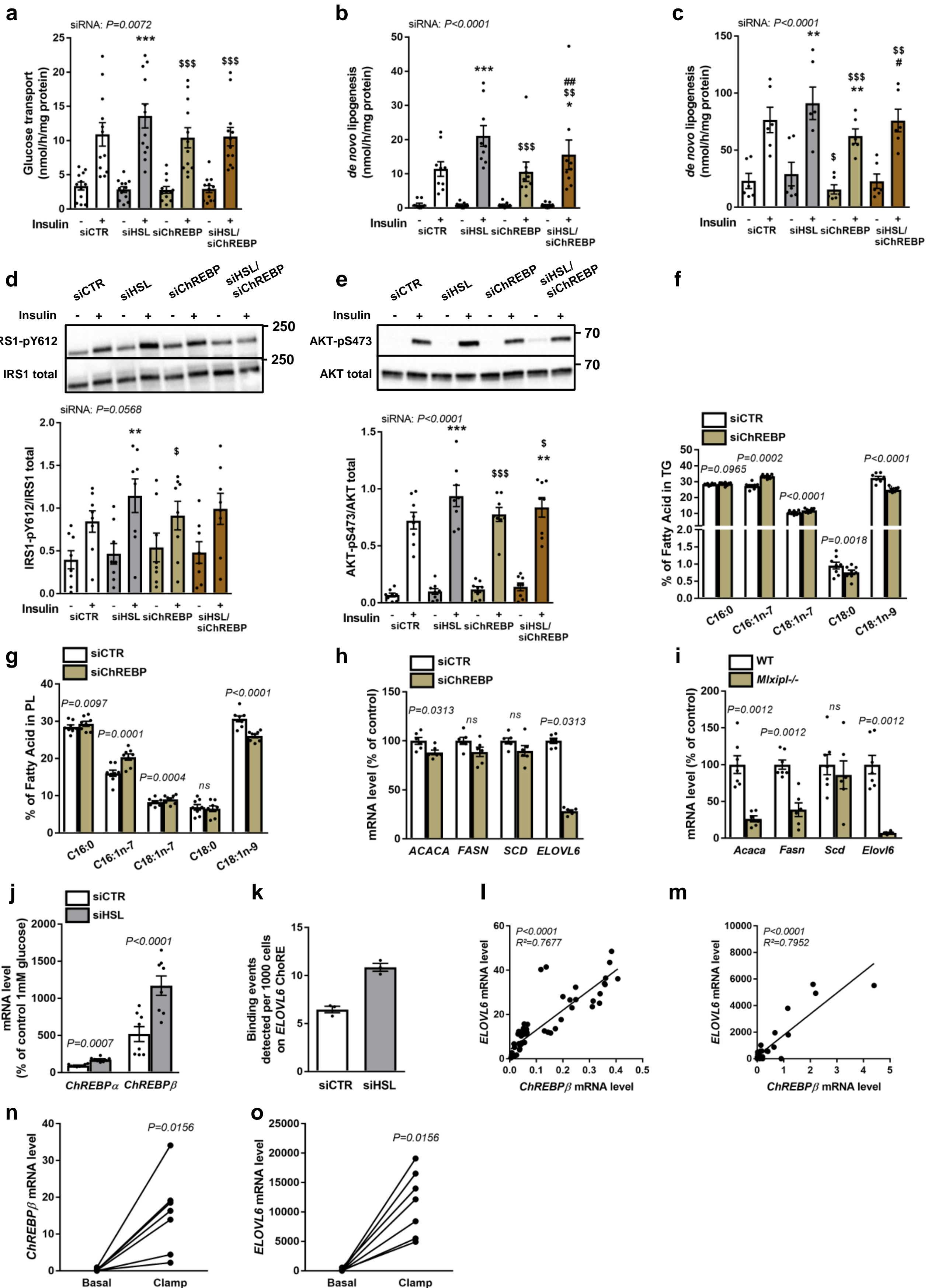

g
ELOVL6 mRNA level
P=0.0061
LE
MS


h
M-value
P=0.0002
Before
After

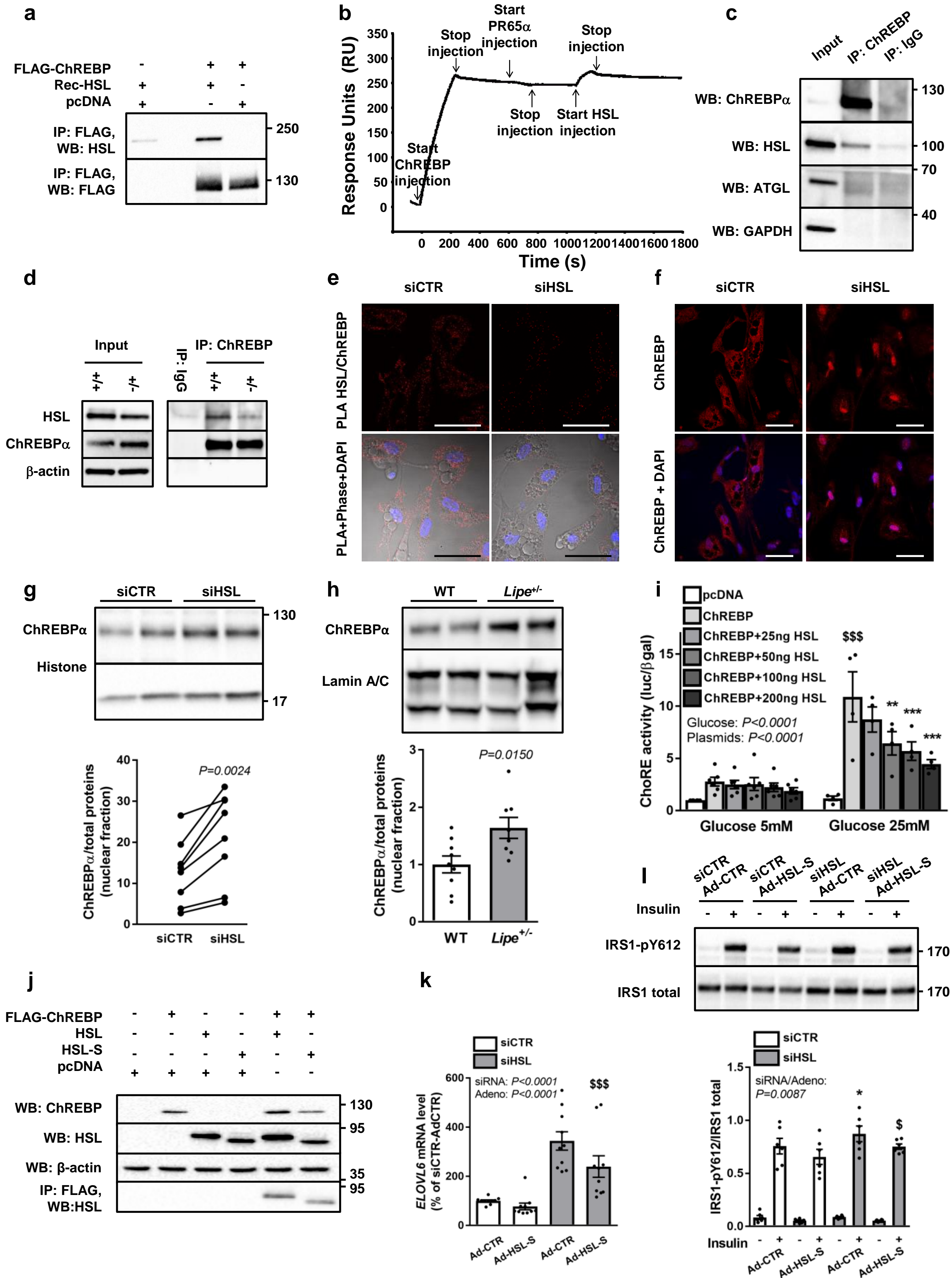

i
ELOVL6 mRNA level
P=0.0001
Before
After



a
siCTR
siELOVL6
% of Fatty Acid in TG
P=0.0313
P=0.0313
ns
P=0.0313
P=0.0313
C16:0
C16:1n-7
C18:1n-7
C18:0
C18:1n-9
b
siCTR
siELOVL6
% of Fatty Acid in PL
P=0.0313
P=0.0313
P=0.0313
P=0.0313
P=0.0313
C16:0
C16:1n-7
C18:1n-7
C18:0
C18:1n-9
c
siCTR
siHSL
siRNA: P=0.0860
ELOVL6i: P<0.0001
(C18:0 + C18:1)/(C16:0 + C16:1)
DMSO
ELOVL6i
DMSO
ELOVL6i
d
siCTR
siHSL
DMSO
ELOVL6i
DMSO
ELOVL6i
Insulin
AKT-pS473
AKT total
70
70
siCTR
siHSL
siRNA/ELOVL6i:
P=0.0138
AKT-pS473/AKT total
Insulin
DMSO
ELOVL6i
DMSO
ELOVL6i
e
siCTR
siHSL
siRNA: P=0.0118
ELOVL6i: P=0.0097
(C16:1+C18:1)/(C18:0+C16:0)
DMSO
SCDi
DMSO
SCDi
f
siCTR
siHSL
DMSO
SCDi
DMSO
SCDi
Insulin
AKT-pS473
AKT total
70
70
siCTR
siHSL
siRNA/SCDi:
P=0.0005
AKT-pS473/AKT total
Insulin
DMSO
SCDi
DMSO
SCDi
g
siRNA/Oleic acid:
P=0.0061
% of Oleic Acid in PL
V
V
V
O100
O500
siCTR
siHSL
siHSL/siELOVL6
h
siCTR
siHSL
siHSL/siELOVL6
V
V
V
O100
O500
Insulin
AKT-pS473
AKT total
70
70
siRNA/Oleic acid:
P=0.0673
AKT-pS473/AKT total
Insulin
V
V
V
O100
O500
siCTR
siHSL
siHSL/siELOVL6
i
P=0.0019
(C18:0 + C18:1)/(C16:0 + C16:1)
Adeno-CTR
Adeno-ELOVL6
j
Adeno-CTR
Adeno-ELOVL6
Insulin
IRS1-pY612
IRS1 total
250
250
Adeno: P=0.0145
IRS1-pY612/IRS1 total
Insulin
Adeno-CTR
Adeno-ELOVL6
k
Phase
GFP
Alexa555-CTxB
Merge
l
P<0.0001
Mobile fraction
Adeno-CTR
Adeno-ELOVL6



a
siRNA: P=0.0072
Glucose transport (nmol/h/mg protein)
Insulin
siCTR siHSL siChREBP siHSL/siChREBP
b
siRNA: P<0.0001
de novo lipogenesis (nmol/h/mg protein)
c
siRNA: P<0.0001
d
IRS1-pY612
IRS1 total
siRNA: P=0.0568
IRS1-pY612/IRS1 total
e
AKT-pS473
AKT total
siRNA: P<0.0001
AKT-pS473/AKT total
f
siCTR
siChREBP
% of Fatty Acid in TG
P=0.0965
P=0.0002
P<0.0001
P=0.0018
P<0.0001
C16:0 C16:1n-7 C18:1n-7 C18:0 C18:1n-9
g
% of Fatty Acid in PL
P=0.0097
P=0.0001
P=0.0004
ns
P<0.0001
h
mRNA level (% of control)
P=0.0313
ns
ns
P=0.0313
ACACA FASN SCD ELOVL6
i
WT
Mlxipl-/-
P=0.0012
P=0.0012
ns
P=0.0012
Acaca Fasn Scd Elovl6
j
siCTR
siHSL
mRNA level (% of control 1mM glucose)
P=0.0007
P<0.0001
ChREBPα ChREBPβ
k
Binding events detected per 1000 cells on ELOVL6 ChoRE
siCTR siHSL
l
P<0.0001
R²=0.7677
ELOVL6 mRNA level
ChREBPβ mRNA level
m
P<0.0001
R²=0.7952
ELOVL6 mRNA level
ChREBPβ mRNA level
n
ChREBPβ mRNA level
P=0.0156
Basal Clamp
o
ELOVL6 mRNA level
P=0.0156
Basal Clamp


a
FLAG-ChREBP
Rec-HSL
pcDNA
IP: FLAG, WB: HSL
IP: FLAG, WB: FLAG
250
130
b
Response Units (RU)
Time (s)
Start ChREBP injection
Stop injection
Start PR65α injection
Stop injection
Start HSL injection
Stop injection
c
Input
IP: ChREBP
IP: IgG
WB: ChREBPα
WB: HSL
WB: ATGL
WB: GAPDH
130
100
70
40
d
Input
IP: ChREBP
IP: IgG
HSL
ChREBPα
β-actin
e
siCTR
siHSL
PLA HSL/ChREBP
PLA+Phase+DAPI
f
siCTR
siHSL
ChREBP
ChREBP + DAPI
g
siCTR
siHSL
ChREBPα
Histone
130
17
ChREBPα/total proteins (nuclear fraction)
P=0.0024
h
WT
Lipe+/-
ChREBPα
Lamin A/C
P=0.0150
i
pcDNA
ChREBP
ChREBP+25ng HSL
ChREBP+50ng HSL
ChREBP+100ng HSL
ChREBP+200ng HSL
ChoRE activity (luc/βgal)
Glucose: P<0.0001
Plasmids: P<0.0001
$$$
Glucose 5mM
Glucose 25mM
j
FLAG-ChREBP
HSL
HSL-S
pcDNA
WB: ChREBP
WB: HSL
WB: β-actin
IP: FLAG, WB:HSL
130
95
35
95
k
siCTR
siHSL
ELOVL6 mRNA level (% of siCTR-AdCTR)
siRNA: P<0.0001
Adeno: P<0.0001
$$$
Ad-CTR
Ad-HSL-S
l
siCTR Ad-CTR
siCTR Ad-HSL-S
siHSL Ad-CTR
siHSL Ad-HSL-S
Insulin
IRS1-pY612
IRS1 total
170
IRS1-pY612/IRS1 total
siRNA/Adeno: P=0.0087