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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 α , independently of lipase catalytic activity, impairs ChREBP α translocation into the
72 nucleus and induction of ChREBP β , 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.

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

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

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

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

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

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

To probe changes in insulin sensitivity upon decreased HSL expression in vivo, we investigated different mouse transgenic models, genetic backgrounds and diets. First, we investigated B6D2/F1 transgenic mice with *Lipe* haploinsufficiency fed with 60% high fat diet¹³. Compared to obese wild type littermates, the mice showed no differences in body weight and fat mass (**Supplementary Fig. 2a,b**). During euglycemic hyperinsulinemic clamp, the glucose infusion rate tended to increase (**Fig. 2a**) while there was no change in glucose rate of disappearance in obese *Lipe* haploinsufficient mice compared with wild type littermates (**Fig. 2b**). Insulin-mediated suppression of hepatic glucose production was improved (**Fig. 2c**). In a second cohort of B6D2/F1 mice fed 45% high fat diet, insulin tolerance was improved while body weight was not modified in *Lipe* haploinsufficient mice compared with wild type littermates (**Fig. 2d, Supplementary Fig. 2c**). In a third cohort of C57BL/6J mice fed 60% high fat diet, we confirmed enhanced insulin sensitivity in *Lipe* haploinsufficient mice as determined by quantitative insulin-sensitivity check index (QUICKI) (**Fig. 2e**). Adipose *Elovl6* gene expression was higher in mice with diminished HSL expression (**Fig. 2f**). As in human adipocytes (**Fig. 1j, Supplementary Fig. 1h**), the induction was more pronounced for *Elovl6* than for other lipogenic genes (**Supplementary Fig. 2d**).

To generate a mouse model with HSL knock down in adipose tissue and unaltered expression in liver, we produced B6D2/F1 mice with zinc finger nuclease-mediated deletion of exon B (**Supplementary Fig. 2e**). The promoter upstream of exon B governs HSL expression in fat cells¹⁸. *Lipe*^{exonB^{-/-}} mice showed decreased expression of HSL in adipose tissue (**Fig. 2g,h**). In liver, the low levels of HSL which are mainly composed of exon A-containing transcripts, were not modified (**Supplementary Fig. 2f,g**). *Lipe*^{exonB^{-/-}} mice fed high fat diet showed improved glucose tolerance (**Fig. 2i**) without alteration of body weight (**Supplementary Fig. 2h**). Adipose *Elovl6* gene expression was higher in these mice compared to wild type

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

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

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

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

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

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

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

phosphatidylserines. These findings were in agreement with in vivo data, where the lack of *Elovl6* in mouse adipose tissue resulted in increased palmitic and palmitoleic acid and decreased oleic acid contents in adipose tissue (**Supplementary Fig. 4e**). The changes in FA composition of PL suggest potential modification in plasma membrane properties. As ELOVL6 mediates the positive effect of HSL gene silencing on insulin signaling (**Fig. 3a,b**, **Supplementary Fig. 3c**), we determined whether this effect was dependent on oleic acid. There are two enzymatic steps between palmitic acid and oleic acid (**Supplementary Fig. 1d**). The first is the elongation of palmitic acid into stearic acid catalyzed by ELOVL6 and the second is the desaturation of stearic acid into oleic acid catalyzed by SCD, a highly active process in fat cells¹⁹⁻²². To investigate the respective contribution of the two steps, specific inhibitors were used. Treatment of human adipocytes with an inhibitor of ELOVL6²³ resulted in the expected changes in FA composition with a decrease of the C18/C16 FA ratio (**Fig. 4c**). Concordant with data obtained using gene silencing (**Fig. 3b**), pharmacological inhibition of ELOVL6 abrogated the enhancement of insulin-induced AKT phosphorylation observed in HSL-deficient adipocytes (**Fig. 4d**). A specific SCD inhibitor²⁴ decreased C16 and C18 FA desaturation (**Fig. 4e**) and had the same effect as the ELOVL6 inhibitor on AKT phosphorylation (**Fig. 4f**). Our data suggest that SCD is necessary but does not play a rate-limiting role as does ELOVL6 in the improvement of insulin signaling induced by HSL inhibition. Accordingly, *SCD* mRNA levels are much higher than *ELOVL6* mRNA levels in human adipocytes (**Supplementary Fig. 4f**). In additional experiments, the content of oleic acid in PL was directly modified by incubation of adipocytes with the FA (**Fig. 4g**). Exposure of HSL and ELOVL6 double deficient adipocytes to oleic acid rescued insulin-induced AKT phosphorylation to levels comparable with that observed in fat cells with diminished HSL expression (**Fig. 4h**). Therefore, the beneficial role of ELOVL6 on insulin signaling in human adipocytes is mediated by modulation of oleic acid content.

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

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

ELOVL6 is a direct transcriptional target of ChREBP²⁷. Adipose ChREBP is a major determinant of systemic insulin action on glucose metabolism²⁸. Therefore, the direct contribution of ChREBP to HSL gene silencing-mediated improvement of glucose metabolism in human adipocytes was evaluated using RNA interference (**Supplementary Fig. 5a**). During dual knockdown of HSL and ChREBP, the beneficial effects on glucose metabolism observed in adipocytes with low HSL expression were diminished. Glucose transport was decreased to control levels and the induction of *de novo* lipogenesis observed in cells with single HSL knockdown was lowered in adipocytes with dual knockdown of HSL and ChREBP (**Fig. 5a,b**). A similar pattern was observed for glucose and acetate carbon incorporation into FA (**Fig. 5b,c**) showing that the upregulation of FA synthesis resulted not

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

Two isoforms of ChREBP have been identified. ChREBP α , which transcriptional activity is regulated by glucose, and ChREBP β , a transcriptionally superactive and unstable isoform which is a direct transcriptional target of ChREBP α ^{28,29}. We characterized the human β -specific exon of *MLXIPL* which extends 29 deoxynucleotides 3' of its mouse counterpart²⁸ (**Supplementary Fig. 5c**). In human adipocytes with siRNA-mediated knock down of HSL, the levels of *ChREBP* transcripts, notably the β isoform, were increased (**Fig. 5j**). Recruitment of ChREBP on the functional carbohydrate response element (ChoRE) in the *ELOVL6* promoter was investigated using chromatin immunoprecipitation assays²⁷. In hMADS adipocytes, more binding events were detected on the *ELOVL6* ChoRE than on positive control regions in *RORC* and *TXNIP*, a well characterized target of ChREBP (**Supplementary Fig. 5d**). ChREBP recruitment onto the *ELOVL6* promoter was markedly enhanced in HSL-deficient compared to control adipocytes (**Fig. 5k**).

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

HSL modifies ChREBP activity in fat cells through protein-protein interaction

As HSL catalyzes one of the rate-limiting steps in fat cell TG hydrolysis, we investigated whether lipolysis per se contributed to the induction of ChREBP. Several lines of evidence suggest that this is not the case. In the culture conditions used to study *de novo* lipogenesis, the release of glycerol and FA in the culture medium was low and was not influenced by HSL gene silencing (**Supplementary Fig. 6a,b**). Adipocytes were then treated with triacsin C, a potent inhibitor of long chain fatty acyl CoA synthetase¹⁴. If FAs were involved, enhanced FA levels due to blockade of FA re-esterification should influence *ChREBP* and *ELOVL6* induction in adipocytes with HSL knock down. However, the upregulation of *ChREBP* isoform and *ELOVL6* mRNA was not influenced by the treatment (**Supplementary Fig. 6c-e**). These data show that the lipolytic activity of HSL does not contribute to the induction of ChREBP.

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

In mouse adipose tissue, co-immunoprecipitation between HSL and ChREBP was diminished in *Lipe* haploinsufficient mice (**Fig. 6d**). In human adipocytes, HSL binding to ChREBP α was reduced in cytoplasm when HSL expression was diminished using siRNA (**Fig. 6e, Supplementary Fig. 7a**). This resulted in modification of ChREBP cellular distribution. Compared to control cells, adipocytes with low HSL expression showed higher immunofluorescence of ChREBP in nuclei indicating that ChREBP α nuclear translocation is facilitated when interaction with HSL is diminished (**Fig. 6f**). Subcellular fractionation confirmed an increased nuclear translocation in human adipocytes and mouse adipose tissues with low HSL expression whereas no significant differences was observed in the cytosolic fraction (**Fig. 6g,h, Supplementary Fig. 7b,c**). In mice, there was no difference in ChREBP α protein content in fat pads of the two genotypes (**Supplementary Fig. 7d**). To evaluate the effect of HSL on ChREBP α transcriptional activity, HEK293 cells were transfected with a vector containing the luciferase reporter gene under the control of a promoter containing functional ChoREs³². Promoter activity increased when cells expressed ChREBP α and decreased when cells co-expressed increasing amounts of HSL (**Fig. 6i, Supplementary Fig. 7e**). The data suggest that HSL binds to ChREBP α and sequesters the transcription factor in the cytoplasm. Upon decrease of HSL expression, HSL-ChREBP interaction is diminished, ChREBP α nuclear translocation is facilitated and its transcriptional activity is enhanced as shown here in reporter gene assays and above in chromatin immunoprecipitation analysis (**Fig. 5k**).

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

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

Discussion

Considering the soaring incidence of diseases characterized by an insulin resistance state, there is a lack of drugs acting on adipose tissue. Its specialized cells, the adipocytes, have great potential to be therapeutically targeted³⁵. Partial inhibition of the fat cell neutral lipase, HSL, alleviates insulin resistance without increasing body weight, two essential requirements for therapeutic interventions¹³ (and present work). Evolution of plasma fatty acid level and variation in insulin sensitivity was dissociated in this model. Here, we deciphered the mechanisms behind HSL inhibition-mediated improvement of glucose metabolism and identified interactions between prototypical metabolic pathways of the adipocyte. We show that, independently of lipolysis and the enzyme catalytic activity, HSL physically interacts with and inhibits the transcription factor ChREBP. ChREBP controls the FA elongase *ELOVL6* catalyzing a limiting step in oleic acid synthesis. The resulting increase in PL oleic acid content modifies plasma membrane properties and improves insulin signaling.

Adipose *de novo* lipogenesis is positively associated with systemic insulin sensitivity^{36,37}. *De novo* lipogenesis is under the control of the glucose-responsive transcription factor, ChREBP. A positive association between insulin sensitivity and adipose ChREBP, notably, the transcriptionally superactive β isoform, has been reported^{16,28,38}. In human adipocytes, we show that knockdown of ChREBP counteracts the beneficial effects of HSL gene silencing on insulin sensitivity. We identify the FA elongase ELOVL6 as the main target of ChREBP β in HSL-deficient adipocytes. In humans, *ELOVL6* expression in fat was lower in insulin resistant than in insulin sensitive subjects in line with previous reports^{16,39}. Of note, in monozygotic twin pairs discordant for type 2 diabetes, adipose *ELOVL6* is markedly lower in the affected twins⁴⁰. Our results from bariatric surgery, a longitudinal intervention improving insulin control of glucose metabolism, also supported the tight link between adipose *ELOVL6* and insulin sensitivity.

ELOVL6 catalyzes a critical step in the elongation of C16 FA^{22,41}. In adipocytes, enhanced ELOVL6 activity favored oleic acid synthesis while ELOVL6 knock down had the opposite effect. Diets rich in olive oil improve insulin sensitivity at adipocyte and whole-body levels^{42,43}. This effect may contribute to the decreased incidence of type 2 diabetes in patients at risk fed Mediterranean diets⁴³. At the cellular level, monounsaturated fatty acids have been reported to protect from the damaging effect of palmitic acid on insulin signaling^{19,26}. As ELOVL6 induced an increase of oleic acid in major classes of PL, we postulated that it may alter plasma membrane fluidity owing to its conformational plasticity^{25,44,45}. The plasma membrane lateral mobility of glycolipids was increased in fat cells overexpressing ELOVL6. We therefore propose that ELOVL6-mediated increase in PL oleic acid content improves fat cell insulin signaling through alteration of plasma membrane properties. In mice, *Elovl6* deficiency impaired white adipose tissue insulin signaling whereas the opposite or lack of

alteration have previously been reported in the liver suggesting tissue-specific differences in ELOVL6-mediated modulation of insulin action^{41,46}.

The role of HSL in human fat cell lipolysis is well established^{14,47}. HSL is a multifunctional enzyme with a broad range of substrates. Besides tri-, di- and monoglycerides, HSL is able to hydrolyze other esters, such as cholesteryl and retinyl esters⁴⁸. As ChREBP activity is influenced by metabolites and other transcription factors in liver, it could be postulated that products of HSL enzymatic activity directly or indirectly influence ChREBP-mediated modulation of gene transcription²⁹. However, although we cannot rule out that, in some conditions, upregulation of ChREBP α protein expression partially contributes to the phenotype of adipocytes depleted in HSL, we bring a solid body of evidence that physical interaction between HSL and ChREBP α controls the intracellular location and activity of the transcription factor in fat cells. ATGL which catalyzes the first step in adipose tissue lipolysis does not interact with ChREBP and does not modulate *de novo* lipogenesis gene expression. Moreover, using a short inactive form of HSL lacking the catalytic site Serine, we could show that the catalytic activity of HSL is dispensable for the interaction with ChREBP and ChREBP-mediated effect on *ELOVL6* expression and insulin signaling. Noteworthy, our data provide a function to this naturally occurring form expressed in human adipose tissue³³. A specific HSL inhibitor was able to diminish the interaction between HSL and ChREBP and enhance adipose *Elovl6* expression in mice. It may be hypothesized that the inhibitor binding to the catalytic pocket induces conformational change partially disrupting HSL-ChREBP interaction. The data suggest that small molecules may be designed and used to disrupt the interaction. Reducing the interaction between HSL and ChREBP favors ChREBP nuclear translocation and its transcriptional activity. This pathway provides a molecular basis to the differential control of *de novo* lipogenesis in liver and adipose tissue. ChREBP is involved in the regulation of *de novo* lipogenesis in the two tissues^{29,49}. However, the pathway is

generally considered as detrimental in the liver as it is activated during the development of fatty liver disease whereas it is seen as beneficial in adipose tissue as the link with insulin sensitivity has been shown both in clinical studies and in mouse models⁵⁰. HSL is expressed at much higher level in fat cells than in hepatocytes. Accordingly, interaction between HSL and ChREBP is not found in human hepatocytes. Alleviation of HSL-mediated inhibition of ChREBP activity may constitute a fat cell-specific mechanism to enhance *de novo* lipogenesis and insulin signaling.

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

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AUTHOR CONTRIBUTIONS

P.M. and M.H. share first authorship. P.M. and M.Ho. performed the majority of in vitro experiments and analyzed data with the contribution of A.Mai., C.G., F.B., B.M., E.R., 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., 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 from mouse models. P.M., S.B., M.M., B.F., A.A., E.Me., C.L., R.R.L., W.S., V.St., P.A., M.R., N.V. and H.V. performed and analyzed in vivo data in human clinical studies. S.C.-B., 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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FIGURE LEGENDS

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

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

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

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

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

Figure 4. Oleic acid content in PL and plasma membrane fluidity mediates ELOVL6 positive effect on insulin signaling. (**a-h**) Experiments were carried out in control (white bars, siCTR), single HSL (grey bars, siHSL), single ELOVL6 (light orange bars, siELOVL6) or dual HSL/ELOVL6-deprived (dark orange bars, siHSL/siELOVL6) hMADS adipocytes. (**a**, **b**) Fatty acid composition in TG (**a**) and PL (**b**) ($n=6$ biologically independent samples per group). (**c-f**) hMADS adipocytes were treated with vehicle (DMSO), $1\mu\text{M}$ 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 μ M (O100) or 500 μ 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 μ 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 ^{\$\$}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*

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

Figure 6. HSL inhibits ChREBP activity through protein-protein interaction. **(a)** Representative image of immunocomplexes between immobilized FLAG-ChREBPα and recombinant HSL (Rec.HSL) (n=3 independent experiments). **(b)** Representative surface plasmon resonance assay sensorgram showing the binding of HSL to ChREBP. Purified ChREBP (220RU) was first injected on a sensorchip with immobilized anti-ChREBP antibody. Following ChREBP binding, PR65α (no signal) and HSL (35RU) were consecutively injected (n=3 independent experiments). **(c)** Endogenous interaction between HSL and ChREBP in human adipocytes (n=3 biologically independent samples per group). Anti-ChREBP antibody was used for immunoprecipitation (IP). Normal Rabbit IgG antibody was used as negative control. **(d)** Endogenous interaction between HSL and ChREBP in white adipose tissue of *Lipe*^{+/-} (+/-) and wild type (+/+) mice (n=4 animals per group). Anti-ChREBP antibody was used for immunoprecipitation. Rabbit IgG antibody was used as 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 length 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 \pm 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**).

METHODS

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

Culture of human adipocytes and in vitro measurements.

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

For primary culture and differentiation of human preadipocytes, subcutaneous adipose tissue samples were obtained from 5 women (age 39 ± 9 years; BMI 28 ± 4 kg/m²) undergoing elective plastic surgery in the abdominal or dorsal region at Rangueil Hospital, Toulouse, France. Adipose tissue was cleaned from blood vessels and fibrous material, minced into pieces and digested in 1 volume of collagenase I (300 units/ml, Sigma) for 90 min in 37 °C shaking water bath. Digested tissue was filtered through 250 µm strainer, diluted with PBS/gentamycin and centrifuged at 1300 rpm for 5 min. Pellet was incubated in erythrocyte

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

HEK293 and HepG2 cell cultures. See Supplementary Methods.

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

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

Plasmid transfection. See Supplementary Methods.

Oleic acid supplementation in human adipocytes. See Supplementary Methods.

Treatments with enzyme inhibitors. For FAS, SCD and ELOVL6 inhibition, hMADS adipocytes were respectively treated with 1 μ M of compound AZ12756122 (ex 117 from WO2008075070A1, synthesized at AstraZeneca), 75nM of A939572²⁴ (Tocris Biosciences) and 1w²³ (provided by AstraZeneca) in culture medium for 48 hours. To study the effect of

bioactive FA on the induction of ChREBP, cells were treated for 8h with 10 μ M of triacsin C (Sigma), an inhibitor of acyl-CoA synthase, in the culture medium.

Gene expression analysis. See Supplementary Methods.

Characterization of human ChREBP β -specific exon. See Supplementary Methods.

Western blot analysis. See Supplementary Methods.

Metabolic measurements. Triacylglycerol hydrolase activity was measured on cell extracts¹⁴. For other metabolic measurements, insulin was removed from culture medium the day before the assay. To determine glucose uptake, cells were incubated 50 min at 37°C with or without 100 nM insulin. Then, 125 μ M of cold 2-deoxy-Dglucose and 0.4 μ Ci 2-deoxy-D-[³H] glucose (Perkin Elmer) per well were added for 10 min incubation. Culture plates were put on ice and rinsed with 10 mM glucose in ice-cold PBS and then with ice-cold PBS. Cells were scraped in 0.05N NaOH, and radioactive 2-deoxy-D-glucose uptake was measured by liquid scintillation counting of cell lysate. To determine glucose oxidation, cells were incubated for 3 h in Krebs Ringer buffer supplemented with 2% BSA, 10 mM HEPES, 2 mM glucose, and 1 μ Ci D-[¹⁴C(U)]glucose (PerkinElmer) with or without 100 nM insulin. A 2x2 cm Whatman 3M paper was placed on top of each well and soaked with 120 μ L NaOH 1N. After incubation, filter-trapped ¹⁴CO₂ was measured by liquid scintillation counting. Medium was acidified with 1M sulfuric acid and medium ¹⁴CO₂ was trapped by benzethonium hydroxide, during 2 h incubation. Benzethonium-trapped ¹⁴CO₂ was measured by liquid scintillation counting. Specific activity was counted and used to determine the quantity of oxidized glucose equivalent. To assess glucose incorporation into FA, cells were then washed twice in PBS and then scraped in STED. Neutral lipids were extracted in methanol/chloroform (1:2). Organic phase was dried under nitrogen and hydrolyzed in 1mL 0.25N NaOH in methanol/chloroform (1:1) for 1 h at 37°C. The solution was neutralized with 500 μ L 0.5N HCl in methanol. FAs and glycerol were separated by adding 1.7 mL chloroform, 860 μ L

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

ELOVL6 activity. Fatty acid elongation activity was measured in crude microsomal extracts from hMADS adipocytes⁴¹. Briefly, cells were washed with PBS, scraped in 3 ml of ice-cold 250mM sucrose, Hepes 20 mM, EDTA 1 mM, pH 7.5 and dounce-homogenized. Homogenate was centrifuged 1000 g at 4°C for 7 min. Supernatant was collected and centrifuged at 2000 g at 4°C for 30 min. Supernatant was collected and centrifuged at 17000 g at 4°C for 1 hour. The resultant pellet was suspended in 50 μl of 100 mM Tris-HCl, pH 7.4 and used for fatty acid elongation activity after determination of protein concentration. ELOVL6 activity was assayed by the measurement of [2- ^{14}C]malonyl-CoA (Perkin Elmer) incorporation into exogenous palmitoyl-CoA⁵³. ELOVL6 inhibitor (1 μM of compound 1w²³) was preincubated 30 min at 37°C with microsomal protein before addition of reaction mixture. Incubation was stopped by adding 0.2 ml of 5M KOH, 10% methanol and saponified at 65°C for 1h. Then the samples were cooled and acidified with 0.2 ml of ice-cold 5N HCl and 0.2 ml of ethanol. Free fatty acids were extracted from the mixture three times with 1 ml of hexane, 2% acetic acid. The pooled hexane fractions were dried under nitrogen and after addition of 3 ml of scintillation cocktail, the radioactivity incorporated was counted. Blanks

were carried out in parallel reactions incubated without microsomal fractions. ELOVL6 activity obtained by subtracting [^{14}C]malonyl-CoA molecules incorporated into fatty acids in the absence of inhibitor to the values in the presence of the ELOVL6 inhibitor.

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

Cellular subfractionation. Nuclear and cytosolic fractions from hMADS adipocytes were prepared using Nuclear Extract Kit (40010) from Active Motif. Cells were rinsed with PBS 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- μ 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 μ 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

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

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

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

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

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

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

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

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

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

Luciferase activity. See Supplementary Methods.

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

Mouse models. Targeted disruption of the *Lipe* gene and generation of *Lipe*^{+/-} mice have been described elsewhere¹³. Before euthanasia, mice were fasted for 24h or refed for 18h supplemented with 20% glucose in drinking water. To create transgenic mice with specific deletion of *Lipe* exon B, mRNA coding for zinc finger nucleases targeting specifically HSL (CompoZr™ Custom Zinc Finger Nucleases, CSTZFN-1KT, Sigma) was injected into pronuclei of one-cell embryos from female B6D2/F1 mice. Homozygous mice (*Lipe*^{exonB-/-}

registered as B6D2-*Lipe*^{em1Land} mice) were obtained. Full description of the model will be published elsewhere. The specific inhibitor of HSL (BAY 59-9435) was synthesized by NoValix (Illkirch, France)⁵⁵. Transgenic mice were fed high fat diet (60% or 45% kcal fat, respectively, D12492 and D12451 from Research Diets) for indicated times. In pharmacological studies, C57BL/6J male mice (12-15 weeks-old, Janvier Laboratories) were treated orally with DMSO or HSL inhibitor (70mg/kg once daily) for 11 days. Eight week-old DBA2/J and C57BL6/J male mice (Charles River) were fed high fat diet (60% kcal fat, D12492 from Research Diets) for 6 weeks before sacrifice. Mice were housed and manipulated according to Inserm guidelines and European Directive 2010/63/UE in the local animal care facility (agreements A 31 555 04 and C 31 555 07). Protocols were approved by the French Ministry of Research following review by local ethical committee (CEEA122).

In studies on ChREBP null mice, 10 to 12 week-old male and female *Mlxipl* global knockout mice⁵⁶ and wild-type littermates were maintained in a 12-h light/dark cycle with water and chow diet (65% carbohydrate, 11% fat, and 24% protein). For fasting-refeeding experiment, mice were either fasted for 24h (fasted group) or refed for 18h on chow diet and had access to drinking water with 20% glucose, following a 24h fast (Refed group). Mice were housed and manipulated according to Inserm guidelines and European Directive 2010/63/UE in the local animal care facility (agreement A751320). Protocols were approved by the French Ministry of Research following review by local ethical committee (CEEA34).

Mice homozygous for a deletion in *Elovl6* and their wild-type littermates were phenotyped on a C56BL6/J background⁵⁷. The research has been regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB).

Gene and protein expression analyses. See Supplementary Methods.

Measurement of fasting glucose and insulin. See Supplementary Methods.

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

Euglycemic-hyperinsulinemic clamp. See Supplementary Methods.

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

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

Hyperglycemic hyperinsulinemic clamp. The 8 participating men were 23 ± 3 years-old (BMI, 23 ± 2 kg/m²). The hyperglycemic hyperinsulinemic clamp was a modification of the hyperglycemic method used by Del Prato et al. combined with the original hyperinsulinemic clamp described by DeFronzo ^{59,60}. For hyperglycemia, the objective was to increase plasma glucose 5.5 mmol/l above fasting level by infusing 20% dextrose in two phases: 1) bolus dose to increase glycemia to the desired target and 2) continuous infusion dose adjusted every 5–10 min according to measured plasma glucose to maintain glycemia at the desired target. To obtain hyperinsulinemia, insulin was co-infused at the rate of 75 mU/m²·min for 180 min. The study was approved by the Ethics Committee of University of Montreal. The volunteers gave their written consent after being informed of the nature, purpose, and possible risks of the study.

Morbidly obese subjects undergoing bariatric surgery. This cohort has in part been described before ⁶¹. In brief, 14 obese women (BMI>35 kg/m²; age, 48 ± 9 years) referred to the

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

Gene expression analysis. See Supplementary Methods.

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

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

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