Dysregulation of amyloid precursor protein impairs adipose tissue mitochondrial function and promotes obesity

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Mitochondrial function in white adipose tissue (WAT) is an important yet understudied aspect of adipocyte biology. Here, we report a role for amyloid precursor protein (APP) in compromising WAT mitochondrial function through a high-fat diet (HFD)-induced, unconventional mis-localization to mitochondria that further promotes obesity. In humans and mice, obese conditions induce substantial APP production in WAT and APP enrichment in mitochondria. Mechanistically, HFD-induced dysregulation of signal recognition particle subunit 54c is responsible for the mis-targeting of APP to adipocyte mitochondria. Mis-localized APP blocks the protein import machinery, leading to mitochondrial dysfunction in WAT. Mice overexpressing adipocyte-specific and mitochondria-targeted APP display increased body mass and reduced insulin sensitivity, along with dysfunctional WAT, owing to a dramatic hypertrophic program in adipocytes. Elimination of adipocyte APP rescues HFD-impaired mitochondrial function with considerable protection from weight gain and systemic metabolic deficiency. Our data highlight an important role for APP in modulating WAT mitochondrial function and obesity-associated metabolic dysfunction.

A better understanding of the processes that cause adipose tissue (AT) dysfunction is essential for understanding the pathophysiology of obesity, and could identify new targets for preventing and treating obesity-associated comorbidities. Weight gain causes an expansion of AT that involves adipocyte hypertrophy, which contributes to impaired adipocyte function and subsequent AT inflammation and fibrosis. Large adipocytes are resistant to insulin’s action on lipolysis and glucose uptake, and show impaired secretion of adiponectin, a hormone that enhances insulin sensitivity. Hypertrophic adipocytes are at increased risk of hypoxia, which drives a fibrotic program that limits healthy AT expansion. Clinical studies have corroborated enlarged adipocytes with AT dysfunction, whole-body metabolic defects and systemic insulin resistance. Increased subcutaneous adipocyte size is a predictor of obesity-related comorbidities, such as type 2 diabetes. Accordingly, targeting adipocyte hypertrophy has the potential to improve metabolic function in people with obesity. However, the driving forces of adipocyte enlargement have yet to be identified.

Another characteristic of metabolically unhealthy adipocytes is mitochondrial dysfunction, which affects adipogenesis, adipokine secretion, lipogenesis and lipolysis. Evidence from human and animal studies show that obesity is associated with AT mitochondrial dysfunction, manifested by reduced mitochondrial DNA (mtDNA) in white AT (WAT), decreased electron transport chain (ETC) gene expression, impaired mitochondrial oxidative capacity and increased levels of reactive oxygen species (ROS). By employing transgenic and tissue-specific-knockout models in rodents, several factors that modulate AT mitochondrial function have been identified, including uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) and mitoNEET.

APP is intensively investigated in the neuroscience field because of its essential contribution to Alzheimer’s disease (AD) pathogenesis through the generation of toxic amyloid β (Aβ) aggregates, potentially causing neurodegeneration. However, very few studies have been conducted to investigate the action of APP in peripheral organs. Notably, APP expression occurs in both mouse and human WAT, where it is substantially induced by high-fat diets (HFD). Zheng et al. noticed that systemic App deficiency leads to a 15–20% reduction in body weight, but the reasons for lighter body mass remain unknown.

Here, we report an unconventional location for APP in adipocyte mitochondria under obese conditions, and an unexpected role of APP in regulating adipocyte mitochondrial function, lipolysis and hypertrophy. Following the observation that APP is upregulated in AT and enriched in adipocyte mitochondria under obesity conditions, we generated adipocyte-specific, doxycycline (Dox)-inducible App-overexpressing and App-knockout mouse models. Enhanced presence of APP in mitochondria impairs adipocyte mitochondrial function, resulting in a suppressed lipolysis and rapid adipocyte hypertrophy. Adipocyte-specific elimination of App maintains mitochondrial respiration, protects mice from HFD-induced adipocyte hypertrophy and promotes systemic metabolic health.
Results
APP is increased in obesity and accumulates in adipocyte mitochondria. We examined the alterations in APP levels in human and mouse WAT, particularly in the context of obesity. We found an inverse relationship between subcutaneous (sWAT) APP mRNA levels and insulin sensitivity in people with obesity, as judged by the glucose infusion rate (GIR) during a hyperinsulinemic–euglycemic clamp procedure (Fig. 1a). In addition, human sWAT APP mRNA levels show positive correlations with body mass (Extended Data Fig. 1a), body mass index (BMI) (Extended Data Fig. 1b), subcutaneous adiposity (Extended Data Fig. 1c), plasma triglycerides (Extended Data Fig. 1d) and fasting insulin levels (Extended Data Fig. 1e), but are inversely correlated with high-density lipoprotein (HDL) cholesterol (Extended Data Fig. 1f). These findings demonstrate that increased sWAT APP expression is associated with obesity, insulin resistance and atherogenic dyslipidaemia in humans. Furthermore, we evaluated public human genomic data resources to gather more evidence demonstrating the clinical relevance of APP changes in human obesity. In a report by Pietilainen et al., global gene-expression analysis (E-MEXP-1425) was performed using sWAT samples from lean and obese monoyzotic twin pairs, and APP levels were substantially increased in the twins with obesity (Fig. 1b). In another study conducted by Lee et al. in Pima Indians (GSE2508)28, 20 of whom were non-obese and 19 of whom were obese, we found the expression of APP in sWAT is higher in obese than in non-obese groups (Fig. 1c). One study (GSE27951)24 that reported on adipocyte size in sWAT demonstrated that APP expression progressively increases with enhancements in mean adipocyte diameter (Fig. 1d).

In agreement with those human studies, we demonstrated that APP mRNA levels are substantially upregulated in both leptin-deficient (ob/ob) mice (Fig. 1e) and HFD-induced obese mice (Fig. 1f). The increase of APP expression is much greater in WAT than in other metabolically active organs examined, such as brown AT (BAT) and liver. We further demonstrated that in both sWAT and epididymal WAT (eWAT), the transcription of App is enhanced specifically in adipocytes, but not in the stromal vascular fraction (SVFs) (Fig. 1g). In addition to long-term (30-week) HFD insults, we also fed mice a HFD for shorter periods, and found that an acute HFD challenge substantially induces App levels in both sWAT and eWAT (Fig. 1h), but not in the brain (Extended Data Fig. 1g). After exposing mice to HFD for 24 h, we noticed an ~100-fold increase of App mRNA levels in sWAT, suggesting that App induction is among the earliest adverse effects of a HFD insult.

The mechanistic details of how higher levels of APP cause cellular changes are not known. To define the function of APP, we explored the intracellular localization of the upregulated APP protein in WAT. Naturally, the majority of APP is targeted to the endoplasmic reticulum (ER) and subsequently enters the secretory pathway25. Surprisingly, we observed that in addition to an increase in the cytoplasmic fractions, APP protein is also substantially enriched in adipocyte mitochondria (Fig. 11). This unconventional localization to mitochondria prompted us to further study the impact of APP on adipocyte mitochondrial function.

APP-overexpressing mice are more sensitive to diet-induced obesity. To directly investigate the role of APP in white adipocytes in a cell-specific manner, we generated a mouse model carrying a tetracycline-responsive element (TRE)-driven and mitochondria-targeted APP transgenic cassette (TRE-mito-APP). By crossing TRE-mito-APP mice with the adiponectin-promoter-driven reverse-tetracycline-dependent transcriptional activator (rtTA) expressing mice (Adipo-rtTA, control, no APP overexpression), we achieved adipocyte-specific, Dox-inducible APP overexpression (Adipo-APP, with APP overexpression) (Fig. 2a). After administering a HFD and Dox to the mice, we assessed App mRNA levels in fat pads and other tissues. There is a substantial induction in App mRNA in sWAT and eWAT (Fig. 2b); however, there is no increase in App expression observed in mesenteric WAT (mWAT) and perirenal WAT (pWAT), or the liver and the brain (Fig. 2b and Extended Data Fig. 2a). APP transgenic mice display substantially higher levels of APP protein in sWAT (Fig. 2c). Additionally, we also validated the substantial increase of APP expression and colocalization with mitochondrial marker TIM23 in the sWAT of transgenic mice (Fig. 2d). The overexpressed APP substantially accumulates in mitochondria, as suggested by a much higher level of APP protein detected in mitochondria purified from APP transgenic mouse sWAT (Extended Data Fig. 2b), and further supported by a substantial localization of APP and TIM23 in the TRE-mito-APP-transfected HEK 293T cells (Extended Data Fig. 2c).

We next evaluated the metabolic consequences of mitochondrial APP in response to a HFD. Body weights started to diverge almost as soon as the transgene was induced by Dox, and reached a difference with statistical significance around week 7 or 8 (Fig. 2e). The body-weight increase was primarily due to an increase in fat mass (Fig. 2f). Concomitant with the obese phenotype, oral glucose tolerance was impaired in APP-overexpressing mice (Fig. 2g). We also measured circulating insulin levels during the oral glucose tolerance test (OGTT) and found that both basal and glucose-stimulated insulin concentrations are markedly increased in APP-overexpressing mice (Extended Data Fig. 2d). Consistent with the results from the OGTT, an insulin-tolerance test (ITT) also revealed decreased insulin sensitivity in APP transgenic mice (Fig. 2h). Beyond the effects on carbohydrate metabolism, we observed a difference in profiles of circulating lipids. Although cholesterol levels remained unchanged, serum triglycerides were substantially elevated in APP transgenic mice (Fig. 2i). Taken together, increased APP in WAT increased HFD-induced body-weight gain, glucose intolerance, insulin resistance and dyslipidaemia.

APP overexpression causes dysfunctional WAT and liver steatosis. To understand how the local APP induction results in profound metabolic changes, we examined the detailed temporal events during AT expansion. sWAT from transgenic mice displays a dysfunctional expansion phenome with significantly enlarged adipocytes and enhanced crown-like structures (Fig. 3a). Similarly, we observed substantially larger adipocyte size and dramatically increased infiltration of inflammatory cells in eWAT (Fig. 3b). Gene-expression analysis validated an enhanced inflammatory program in both sWAT and eWAT after APP overexpression (Fig. 3c,d). Although genes encoding anti-inflammatory (M2-like) macrophage markers, Il10, Mrcn1 and Clec10a, are unaffected, generic macrophage signature genes Adgre1 and Il6 are substantially elevated; genes encoding pro-inflammatory (M1) macrophage markers, such as Ifng, Tnfa and Nos2, are highly induced in sWAT upon APP overexpression. The eWAT of APP transgenic mice also demonstrates an enhanced pattern of proinflammatory-gene expression. Obesity is frequently associated with AT inflammation that is linked to increased AT fibrosis4. Thus, we also probed the expression of factors involved in AT fibrosis. The major driver for AT fibrosis, Hif1α, was substantially upregulated at the transcriptional level in both the sWAT and eWAT of APP transgenic mice (Fig. 3c,d), further highlighted by substantially increased expression of Hif1α target genes, such as Coll1a1, Col3a1 and Col6a1 in sWAT (Fig. 3c), as well as Lox, Col3a1 and Col6a1 in eWAT (Fig. 3d). We also tested circulating adiponectin levels and found that APP transgenic mice display substantially lower levels of adiponectin in plasma (Fig. 3e), highlighting the influence of AT dysfunction as a systemic mediator of metabolic health. Highly inflammatory and fibrotic AT is characteristic of dysfunctional fat, usually related to unfavourable changes in the liver. Adipo-APP transgenic mice rapidly develop liver steatosis, while control mice remain mostly normal.
protected from HFD-induced lipid accumulation (Fig. 3f). Lipid profiling further showed a substantial elevation in liver triglyceride levels (Fig. 3g). By uncovering significantly elevated AT inflammation/fibrosis and decreased adiponectin, we linked adipocyte-specific APP overexpression to a dysfunctional AT expansion, which led to impaired whole-body metabolism.

**APP leads to adipocyte hypertrophy by impairing lipolysis.** The driving force for AT dysfunction after APP induction is unclear. Next, we took advantage of the Dox inducibility of the APP transgenic mouse model to examine the initial changes in AT by subjecting mice to acute Dox feeding. With the acute induction, APP-overexpressing sWAT displays a rapid enlargement in adipocyte size (Fig. 4a), yet without a substantial difference in total body weight. Not surprisingly, the average adipocyte size in sWAT from control mice remains stable (Extended Data Fig. 3a). This demonstrates that APP overexpression by itself, even in the absence of HFD, is sufficient to induce adipocyte hypertrophy. In addition, the presence of Perilipin 1 is largely maintained, suggesting that adipocytes stayed viable (Fig. 4b). By performing image-based quantitative analysis to map the distribution of adipocyte cell size for each time point, we saw a marked and progressive shift from smaller to larger adipocytes in sWAT over time (Fig. 4c). The increase in average adipocyte size starts within 24 h of APP induction, and continues to progress from day 2 until day 14 (Fig. 4d). In contrast to sWAT, we did not observe a comparable increase in adipocyte size in eWAT (Extended Data Fig. 3b), suggesting that sWAT is the primary tissue affected upon APP overexpression. Indeed, APP expression is substantially higher in sWAT than in eWAT (Fig. 2b).

We aimed to dissect the mechanism(s) underlying how adipocytes can undergo such a rapid onset of hypertrophy upon APP induction. Mechanistically, adipocyte size is controlled by the balance between lipid storage and mobilization, which is mainly driven by lipogenesis and lipolysis. We did not find any substantial changes for genes that are critical for fatty-acid synthesis in sWAT after acute induction of APP (Extended Data Fig. 3c–f),...
suggesting that de novo lipogenesis is not the primary reason for APP-induced adipocyte hypertrophy. Thus, we investigated the impact of APP on lipolytic processes. The increase in serum non-esterified fatty acid (NEFA) and free glycerol levels in APP-overexpressing mice is blunted upon injection of the selective β3-adrenergic receptor agonist CL-316,243 (Fig. 4e). In accordance with this, we confirmed that sWAT fat pads taken from APP transgenic mice demonstrate an impaired response to forskolin-induced NEFA and glycerol release (Fig. 4f). Furthermore, we noticed that while control mice show enhanced circulating insulin levels, APP-overexpressing mice fail to trigger a marked release of insulin in response to CL-316,243 (Fig. 4g). In addition to functional readouts in lipolysis-related gene transcription, and demonstrated that expression levels for genes encoding critical enzymes involved in fatty-acid breakdown are substantially decreased, such as Lipe (encodes hormone-sensitive lipase, HSL), Pnpla2 (encodes adipose triglyceride lipase, ATGL) and Pnpla3; meanwhile, Plin1 and Plin5, which encode proteins that negatively control adipocyte lipolysis, are markedly upregulated (Fig. 4h).

Notably, as a key rate-limiting factor in lipolysis, Lipe displays a time-dependent downregulation after APP induction (Fig. 4h). At the protein level, APP overexpression not only decreases total HSL expression, but also attenuates the phosphorylation of HSL (Fig. 4i, j), in support of HSL suppression having an important role in APP-mediated inhibition of lipolysis. We conclude that acute APP induction in adipocytes causes hypertrophy of sWAT at least in part due to impaired stimulated lipolysis, though we cannot rule out other mechanisms during regular feeding and fasting conditions in these mice.

**APP causes mitochondrial dysfunction, owing to defective protein import.** To test whether the inhibited adipocyte lipolysis by APP is associated with mitochondrial dysfunction, we performed a time-course study to investigate the impact of APP induction on mitochondrial respiration in adipocytes. APP overexpression in adipocytes dramatically impairs mitochondrial function in a time-dependent way (Fig. 5a). Notably, the mitochondrial dysfunction in sWAT happens within 1 d after Dox treatment, in line with APP-induced rapid adipocyte enlargement. We also performed mitochondrial-respiration assays on adipocytes differentiated in vitro, and consistent with the ex vivo decline in mitochondrial respiration, we observed a substantial impairment in basal oxygen consumption, ATP-linked respiration and maximal respiratory capacity in APP-overexpressing adipocytes (Extended Data Fig. 4a).

In addition, adipocytes derived from APP transgenic mice have a significantly diminished membrane potential, as judged by the loss in mitochondrial fluorescence in the presence of TMRE (Extended Data Fig. 4c). Further, APP overexpression results in a decline in ATP production in vitro (Extended Data Fig. 4b). To demonstrate that the mitochondrial dysfunction in adipocytes influences whole-body energy expenditure, we performed metabolic cage studies. APP transgenic mice have lower levels of oxygen consumption and CO₂ production during both light and dark cycles (Fig. 5b and Extended Data Fig. 4d, e). Although the respiratory exchange ratio (RER) is...
identical between genotypes (Extended Data Fig. 4f), energy expenditure in APP-overexpressing mice is substantially suppressed (Extended Data Fig. 4g). In addition to functional changes, we also assessed the morphological alterations in adipocyte mitochondria. Mitochondria in APP-overexpressing adipocytes show elongation, and typical mitochondrial cristae structures disappear (Fig. 5c), consistent with APP impairing mitochondrial respiration.

How does APP enhancement cause dysfunctional mitochondria? At the transcriptional level, we found that the majority of mitochondrial components, such as Cysc, Atp6 and Atp8, and mitochondrial biogenesis regulators Tjaml, Nrf1 and Nrf2 were substantially increased, perhaps as a counter-regulatory response to mitochondrial dysfunction (Fig. 5d). We additionally found that APP overexpression does not lead to changes in mtDNA copy number (Fig. 5e). We conclude that APP-mediated mitochondrial dysfunction is not related to a transcriptional phenomenon. Previous reports suggested that the accumulation of amyloid-β 40/42 (Aβ 40/42) has a toxic role in multiple aspects of neuronal mitochondrial function. However, we did not observe an accumulation of Aβ levels in isolated mitochondria after APP induction (Extended Data Fig. 4h), suggesting that in our adipocyte-specific APP transgenic model, Aβ 40/42 deposition is not the major mediator of mitochondrial dysfunction.

Two additional studies have provided further insights through linking full-length APP with mitochondrial dysfunction. These groups demonstrated that in people with AD, full-length APP is observed in mitochondria, and accumulation of APP in the mitochondrial importation channels blocks protein import since APP is likely to clog the import machinery; this is due to one domain that is rich in acidic amino acids (residues 220–290). Although these observations are intriguing in neurons, such a mechanism has never been explored in any peripheral tissues. Does APP also induce impaired protein import into adipocyte mitochondria? Crucial components of the ETC, including ATP5A (complex V), UQCRQC2 (complex III), SDHB (complex II) and NDUF8 (complex I) are significantly suppressed by APP overexpression (Extended Data Fig. 4i). All these proteins are encoded by nuclear genes and require transport through the mitochondrial protein importing machinery. However, we did not observe a transcriptional change in genes encoding these key ETC components (Extended Data Fig. 4j). Combining these results, we show that even though no changes in nuclear-encoded...
Fig. 4 | APP overexpression leads to adipocyte hypertrophy by impairing stimulated lipolysis. a–d. Rapid enlargement of subcutaneous adipocytes in APP transgenic mice subjected to acute Dox (600 mg per kg (diet weight)) induction for 0, 1, 2, 5, 7 and 14 d. Representative images of H&E staining (a) and perilipin-1 (green) immunofluorescence staining (b) in sWAT sections from different time points, chosen from two independent experiments (scale bars, 161 μm); cell-count distribution according to adipocyte cell size at different time points (c); and average adipocyte size in sWAT at different time points (d). n = 3 mice per time point. e–g. Both control and APP transgenic mice with expression induced by 1-week Dox 600 mg per kg (diet weight) feeding were subjected to in vivo and ex vivo lipolysis analysis: serum NEFA (left), free glycerol (right) (e) and insulin levels (f) at different time points in both groups after β3-adrenoceptor agonist CL-316,243 (1 mg per kg (body weight)) injection, n = 8 mice per group. f. NEFA (left) and free glycerol (right) levels in the media obtained from ex vivo cultured sWAT fat pads at different time points of 10 μM forskolin incubation, n = 12 tissues per group. h. Lipolytic gene expressions in APP-overexpressing and control mice fed Dox 600 mg per kg (diet weight) for 7 d (left, n = 3 mice per group) and a time course of Lipe (gene name for HSL) transcription in sWAT of APP transgenic mice after Dox induction (right, n = 3 mice per time point). i. Representative immunoblot analysis of phosphorylated HSL (p-HSL) and total HSL (t-HSL) levels in the sWAT fat pads from both groups after ex vivo lipolysis assays, with images chosen from three independent experiments (i); quantification for relative p-HSL and t-HSL expression (j), n = 4 tissues per group. Data are shown as mean ± s.e.m. of biologically independent samples. One-way ANOVA followed by a Tukey post-test (d, right panel in h); two-way ANOVA followed by a Tukey post-test (e–g); two-tailed Student’s t-test (left panel in h). See also Extended Data Fig. 3.
Fig. 5 | APP impairs adipocyte mitochondrial function, owing to defective mitochondrial protein import. a, Ex vivo mitochondrial respiration at different time points in sWAT fat pads from APP transgenic mice given Dox 600 mg per kg (diet weight). Oxygen consumption rate (OCR) at basal level and post oligomycin (Oligo), FCCP and rotenone/antimycin-A injection is shown. n = 5 tissues each time point. b, Oxygen consumption (VO2, left) and CO2 production (VCO2, right) measured from indirect calorimetry of control and APP-overexpressing mice under 1-week HFD Dox challenge. Scale bars, 1,000 nm. Images were chosen from two independent biological samples. c, Electron microscopy (EM) images for sWAT sections from both groups under 1-week Dox 600 mg per kg (diet weight) induction. Scale bars, 500 nm. Images were chosen from three independent biological samples. d, Mitochondrial-function-related gene expression (d) and mtDNA copy number (e) in 1-week Dox-fed control and APP transgenic mice. For mtDNA copy number, fold change is normalized to the control with the highest copy-number value. n = 6 mice per group. Images were chosen from two independent biological samples. e, Mitochondrial protein import (f) and pOTC import (g) in isolated mitochondria from sWAT fat pads from APP transgenic mice given Dox 600 mg per kg (diet weight). Arrowheads, dark contrast in the cristae. Scale bars, 0.5 μm. Images were chosen from two independent biological samples. f, Representative autoradiography image (left) and graphical representation (right) of pOTC import assessed in isolated mitochondria (incubation for 30 min) from sWAT control or APP transgenic mice fed for 2 weeks with Dox. Each reaction had 25% of [35S]pOTC loaded as input; 0.4 mg ml−1 protease K (Pro K) was added to digest unimported pOTC proteins; 40 μmol l−1 FCCP inhibits pOTC import via depolarizing the mitochondria (negative control). n = 6 mice per group. Images were chosen from three independent experiments. g, EM images for differentiated adipocytes from SVF isolated from control and APP–APP+ transgenic mice (right). Images are chosen from three independent experiments. h, Representative western blotting image for APP in mitochondrial sub-localization of purified mitochondria from sWAT of control and APP transgenic mice. PDH-E2, mitochondrial matrix; TIM23, inner mitochondrial membrane (IMM); cytochrome C, intermembrane space (IMS); VDAC, outer mitochondrial membrane (OMM). i, Representative western blotting image of co-immunoprecipitation (IP) analysis using either IgG or anti-APP antibody incubated with isolated mitochondria from APP transgenic mice fed a 2-week Dox diet. The input (20% of lysate) and IP samples are subject to immunoblot (IB) with APP, TOM40 and TIM23 primary antibodies. Images are chosen from three independent experiments in h and i. j, A schematic of the proposed clogging model. See also Extended Data Figs. 4 and 5.
mitochondrial gene transcription are observed, the levels of key components located in mitochondria are significantly decreased, supporting the notion that APP decreases mitochondrial protein import. We then moved forward to directly validate that APP mis-targeting to mitochondria inhibits adipocyte mitochondrial protein import by taking advantage of a well-established in vitro protein import assay. This assay utilizes a radio-labeled ornithine carbamylase precursor (pOTC) protein to test the efficiency of protein import in isolated mitochondria. By incubating [35S]pOTC with mitochondria isolated from sWAT, we demonstrated that APP overexpression substantially reduces the kinetics of pOTC import, as judged by a 46% reduction in pOTC import (Fig. 5f). In vivo, APP transgenic mice show increased amounts of COX5A precursor accumulation caused by mitochondrial protein import defects (Extended Data Fig. 4k). To further establish that APP accumulation in mitochondria causes deficient protein import, we performed three independent experiments to refine the sub-mitochondrial localization of APP. The first line of evidence arose from our experiments using the ascorbate peroxidase 2 (APE2) staining system. Upon harvesting differentiated adipocytes from APP-APEX2 transgenic mice, we observed plenty of APEX2-positive staining across mitochondrial outer and inner membranes, suggesting that when APP targets mitochondria, the protein resides close to the mitochondrial protein import machinery (Fig. 5g). We also examined the in vivo mitochondrial sub-localization of APP through biochemical sub-fractionation and observed that, after its mis-targeting to mitochondria, APP is distributed in the outer membrane, intermembrane space and inner-membrane fractions, with no signal in the matrix (Fig. 5h). In addition to the physical presence of APP across mitochondrial outer and inner membranes, we further performed immunoprecipitation assays to show that APP robustly binds to TOM40 and TIM23, the key subunits belonging to translocases of outer membrane and inner membrane, respectively (Fig. 5i). The strong interaction between APP and TOM40/TIM23 may form the molecular basis for the APP clogging action in the protein import pathway, thereby suppressing the pOTC and other nuclear-encoded proteins translocating into adipocyte mitochondria. Collectively, upon APP induction, adipocytes develop mitochondrial dysfunction, and one of the underlying reasons is a blockade of protein import due to clogging of the import channels by APP (Fig. 5j).

SRP54c dysregulation accounts for APP mis-targeting to mitochondria. The driving force responsible for mis-targeting APP to adipocyte mitochondria under obese conditions remains unclear. A recent study by Costa et al. identified an unanticipated role for the yeast signal recognition particle (SRP) in protein-targeting efficiency and specificity, showing that auxin-induced SRP loss causes conventional ER-targeted transcripts to be mis-targeted into mitochondria, thus leading to rapid mitochondrial defects. We explored whether mis-localization of APP is mediated through SRP dysregulation. Examining expression levels of different SRP components in WAT of obese mice, we identified that one SRP subunit, SRP54c, stands out as specifically and massively upregulated in sWAT and eWAT upon prolonged HFD feeding (Extended Data Fig. 5a). Interestingly, even under an acute HFD challenge, SRp54c transcripts show a consistently increased expression pattern (Extended Data Fig. 5b) similar to App transcripts (Fig. 1b). We indeed demonstrated that sWAT expression levels of SRp54c and of App mRNA are substantially correlated (Extended Data Fig. 5c), consistent with the idea that SRP54c is involved in regulating APP in adipocytes. To further dissect the in vivo function of SRP54c, we employed an adipocyte-specific, Dox-inducible overexpression model of SRP54c (SRp54c Tg) (Extended Data Fig. 5d). We confirmed the tissue specificity and inducibility of the SRp54c transgenic mouse model by observing a substantial induction of SRp54c mRNA selectively in sWAT, but not in the liver (Extended Data Fig. 5e). We hypothesized that SRP54c-overexpressing mice would effectively mimic the imbalance of SRP subunits under diet-induced obesity and possibly affect the APP localization. Indeed, Extended Data Fig. 5f demonstrates that 1 week of overproduction of SRP54c in adipocytes is sufficient to induce mis-targeting of APP into mitochondria, leading to a 6-fold increase of APP accumulation in mitochondria in Srp54c transgenic mice without affecting total APP levels. By rapidly and dramatically inducing the mis-localization of APP, Srp54c transgenic mice also display a 33% impairment in pOTC import compared with that in control mice (Extended Data Fig. 5g), phenocopying the inhibition effects on mitochondrial protein import seen in APP transgenic mice. Metabolically, we observed that SRP54c overexpression results in enhanced body-weight gain under a Chow diet (Extended Data Fig. 5h) and accelerated onset of glucose intolerance at earlier time points (Extended Data Fig. 5i). To gain further mechanistic insights into how overproduced SRP54c contributes to adipocyte mitochondrial dysfunction, we subjected highly purified mitochondria to unbiased proteomic analysis through mass spectrometry. After retrieving the data, we focused on the 36 proteins uniquely detected in mitochondria from sWAT of SRp54c Tg mice. Among these, a large portion (31%) of the polypeptides belongs to the secretome (Extended Data Fig. 5j). Notably, we observed a prominent presence of APP (Extended Data Fig. 5j), suggesting that mis-targeting of APP has an important role mediating the mitochondrial dysfunction effects by SRP54c. Several additional proteins containing well-defined ER-targeting sequences are also found in these mitochondrial fractions, such as AGPAT-1, CYP2D22, EPHX-1, GPAT-3 and Napsin-A. Collectively, our data demonstrate that HFD induces a dysregulation of SRP54c, which drives APP mis-targeting, and the excessive SRP54c is sufficient to phenocopy APP transgenic mice. This is the first set of observations that recognizes SRP54c as a key mediator responsible for mis-targeting of APP into mitochondria under obese conditions, triggering the subsequent profound metabolic dysfunction.

**App Ako protects from obesity and enhances mitochondrial function.** Our gain-of-function studies suggest that deleting APP in adipocytes may exert a protective role against HFD-induced obesity and insulin resistance. Thus, we first determined whether halting the overproduction of APP through removal of Dox would reverse the metabolic dysregulation in APP transgenic mice. We demonstrated that by removing Dox, mice gain weight substantially more slowly than mice continuously exposed to HFD and Dox diets (Extended Data Fig. 6a). Without the APP overexpression, adipocyte mitochondrial respiration recovers, as judged by analysis in ex vivo sWAT (Extended Data Fig. 6b) and in isolated mitochondria (Extended Data Fig. 6c). In addition, OGTTs and ITTs both show an improvement in glucose intolerance and insulin resistance without APP overexpression. In contrast, mice continuing to overexpress APP show impaired glucose metabolism and insulin resistance (Extended Data Fig. 6c,d). Importantly, these changes are not due to the removal of Dox, since control mice under the same conditions do not show any of these changes upon removal of Dox; they have identical body-weight gain, unchanged mitochondrial respiration and comparable glucose tolerance as assessed by an OGTT (Extended Data Fig. 6e–h). We observed that mice without Dox have smaller adipocytes (Extended Data Fig. 6i), suggesting that hypertrophy of sWAT adipocytes is attenuated upon cessation of APP overexpression. We noticed that sWAT from mice withdrawn of Dox diets show fewer inflammatory cells. These improvements in AT upon withdrawing Dox trigger improvements in the liver, which displays reduced lipid accumulation (Extended Data Fig. 6j). In the same experimental settings, control mice show similar adipocyte size and inflammatory-cell infiltration in sWAT as well as identical liver steatosis with or without Dox feeding (Extended Data Fig. 6i,j). Thus, upon eliminating APP overexpression in adipocytes...
**Fig. 6 | App AKO protects mice from obesity.** a, Schematic illustration of the adipocyte-specific, Dox-inducible App-KO mouse model. b, c, Validation of App deletion in App AKO mice compared with control mice (Appfloxflox) fed a 4-week HFD plus 4-week HFD and Dox (600 mg per kg (diet weight)). b, App mRNA levels in different tissues from Appfloxflox and App AKO mice (n = 4 mice per group). c, Representative western blotting image (top) for APP protein levels in sWAT and its quantification (bottom, n = 4 mice per group). Images are representative of three independent experiments. d, h, Both Appfloxflox and App AKO mice fed a 4-week HFD plus 4-week HFD and Dox were subjected to metabolic analyses. d, Relative body weight over 8 weeks (n = 8 mice per group). e, f, Glucose levels at different time points, as determined by OGTT (e) and ITT (f) (n = 8 mice per group). g, Relative body weight of the mice over 12 weeks of HFD and Dox feeding. h, Glucose levels at different time points of OGTT assays (n = 8 mice per group in g and h). i–n, Inflammatory and fibrotic phenotypes in sWAT and eWAT from Appfloxflox and App AKO mice fed a 4-week HFD plus 4-week HFD and Dox. Representative H&E staining images for sWAT (i) and eWAT (l). Representative trichrome (TC) staining images for sWAT (j) and eWAT (m); quantification of inflammation- and fibrosis-related gene expression in sWAT (k) and eWAT (n) from mice in both groups (images are chosen from four independent experiments). Scale bar, 100 μm. o, Circulating adiponectin immunoblot (left) and quantification (right) in both groups. n = 4 mice per group. p, Representative H&E staining of liver tissues from both groups (images are chosen from four independent experiments). Scale bars, 100 μm. Data are shown as mean ± s.e.m. of biologically independent samples. Two-tailed Student’s t-test (b,c,k,n,o); two-way ANOVA followed by a Tukey post-test (d–h). See also Extended Data Figs. 6 and 7.
by removing Dox, we demonstrate that mitochondrial function is recovered, functional adipocytes are restored and a significantly improved whole-body metabolism is seen. To directly examine the impact of endogenous APP on diet-induced obesity and insulin resistance, we eliminated App inductively and specifically in adipocytes (App AKO) (Fig. 6a). We validated the inducibility and tissue specificity of App elimination by uncovering a substantial decrease in App mRNA levels in both sWAT and eWAT from App AKO mice (Fig. 6b). Notably, App transcripts are also remarkably downregulated in the BAT of App AKO mice, while its expression is unaffected in the liver (Fig. 6b). We further confirmed that App protein is substantially reduced in the App AKO mouse sWAT (Fig. 6c). Upon demonstrating a substantial reduction of App selectively in adipocytes, we moved forward to investigate the impact of adipocyte-specific App depletion on whole-body metabolism under a HFD challenge. In contrast to the obese phenotype observed for APP-overexpressing mice, App AKO mice show reduced weight gain (Fig. 6d). We noticed an immediate divergence of the weight gain after adding Dox. App AKO mice are not only leaner, but also maintain better glucose tolerance and higher insulin sensitivity, as determined by both OGTT (Fig. 6e) and ITT (Fig. 6f), respectively. We further speculated that a prolonged HFD/Dox exposure allowing a significant induction of mitochondrial dysfunction in adipocytes could fully uncover the protective role of App depletion from obesity. Thus, we performed a 12-week HFD and Dox feeding experiment on control and App AKO mice. We found that App AKO mice are substantially protected from HFD-induced body-weight gain (Fig. 6g). App AKO mice also display much better glucose tolerance at later time points (Fig. 6h), and maintain drastically lower circulating insulin levels during OGTT (Extended Data Fig. 7a), suggesting they have an insulin-sensitive phenotype, which is further validated by a substantial improvement in ITT (Extended Data Fig. 7b).

To strengthen our conclusion and determine the tissue(s) responsible for changes in insulin sensitivity, we performed hyperinsulinemic–euglycemic clamp studies in both the APP transgenic (App Tg) and App AKO mouse models. Consistent with our previous results, we showed that APP overexpressing mice gain substantially more body weight compared to control and App AKO mice (Extended Data Fig. 7c). During the clamp studies, we maintained clamped glucose levels at ~150 mg dl⁻¹ in all three groups (Extended Data Fig. 7d). The GIR needed to maintain euglycaemia is lower in APP transgenic mice and dramatically higher in App AKO mice (Extended Data Fig. 7e). Basal endogenous glucose output is more efficiently suppressed in App AKO mice, but only shows a trend towards an increase in APP transgenic mice (Extended Data Fig. 7f), suggesting that the liver is not a major organ contributing to whole-body insulin-sensitivity changes. We then measured uptake of radiolabeled glucose (2-deoxy-D-glucose, 2-DG) in different metabolic tissues and demonstrated that in both sWAT and soleus muscle, APP transgenic mice display a greatly diminished insulin-stimulated glucose uptake. In contrast, App AKO mice show substantially enhanced glucose uptake (Extended Data Fig. 7g). To complement the clamp studies, we examined the response of individual metabolic tissues to insulin, as assessed by the phosphorylation of Akt (p-Akt). p-Akt levels are clearly lower in sWAT, eWAT and soleus muscle in APP-overexpressing mice than in control mice; in contrast, App AKO mice show an enhanced p-Akt signal upon insulin stimulation in sWAT, eWAT and muscle (Extended Data Fig. 7h–i).

We observed a remarkable improvement in App AKO mice in sWAT histology. These mice display significantly decreased numbers of hypertrophic adipocytes (Fig. 6i), and reduced inflammatory-cell infiltration and fibrotic areas (Fig. 6j). We further validated that App AKO mice are protected from sWAT inflammation and fibrosis by measuring expression levels of some critical genes involved in these processes (Fig. 6k). Similarly, we showed that in eWAT of App AKO mice, the average adipocyte size remains identical to that in control mice, but a dramatic reduction in inflammatory cell infiltration is seen (Fig. 6l). The healthier phenotype is also supported by decreased fibrosis (Fig. 6m). At the transcriptional level, markers for AT inflammation and fibrosis are substantially downregulated in eWAT of App AKO mice (Fig. 6n). In agreement with the overall insulin-sensitive phenotype, App AKO mice show elevated circulating adiponectin levels (Fig. 6o), reflecting better functionality of adipocytes and an enhanced whole-body metabolic homeostasis. Accompanying the morphological and functional improvements in AT, hepatic lipid accumulation is significantly attenuated through the adipocyte-specific depletion of App (Fig. 6p), further underlining the notion that App AKO mice maintain functional AT and consequently improve whole-body metabolism.

Next, we investigated whether the protective effects against diet-induced obesity in App AKO mice are related to improved adipocyte mitochondrial function. The transcription of genes involved in mitochondrial biogenesis and function are largely unaffected (Fig. 7a–c). In contrast to APP-overexpressing mice, we observed an enhancement in pOTC import activity due to adipocyte-specific deletion of App, as suggested by a 50% increase in pOTC import (Fig. 7d). We further examined ex vivo mitochondrial respiration and uncovered a substantially augmented mitochondrial functionality in App AKO mice by showing increased basal respiration and FCCP-induced maximal respiratory capacity in sWAT fat pads (Fig. 7e), as well as enhanced respiration in isolated mitochondria (Fig. 7f). Then, we investigated whether adipocyte App deletion can enhance lipolytic processes. Upon injection of CL-316,243, we noticed that elimination of App dramatically induces lipolysis, as indicated by a substantial enhancement in serum NEFA and free glycerol levels (Fig. 7g). Consistently, we also confirmed that sWAT fat pads taken from App AKO mice demonstrate an augmented response to forskolin-induced NEFA and glycerol release (Fig. 7h). Taken together, the lack of App sustains fully functional adipocytes and preserves metabolic function system wide, likely driven by improvements along the axis of mitochondrial function and lipolysis.

Discussion
We demonstrate that APP, a critical modulator for AD in the brain, also contributes to adipocyte dysfunction during weight gain and obesity. The potential clinical relevance of the findings from our rodent models is supported by our findings that obesity, insulin resistance and dyslipidaemia are associated with upregulation of APP gene expression in AT of people with obesity. Consistently, Puig et al. report that App-deficient mice show improved glucose tolerance and reduced insulin resistance upon HFD feeding. Mutant APP is shown to exacerbate insulin resistance and macrophage infiltration in HFD-challenged AT. However, Czeczor et al. suggest that systemic App-knockout mice develop glucose intolerance under HFD, with a mechanism linking it to impaired insulin secretion from β-cells. APP is expressed in many different cell types, such as neuronal cells, immune cells, β-cells and adipocytes. The interpretation of the metabolic characterization of congenital and ubiquitous loss-of-function models is therefore very challenging. Our present study utilizes mouse models with adipocyte-specific manipulations, which provide direct evidence uncovering the crucial role of APP in adipocyte physiology and obesity.

Few studies have investigated the relationship between full-length APP and mitochondrial function. APP accumulates in mitochondria in neuronal cells and impairs capacity to import nuclear-encoded components by clogging import machinery, and APP is highly enriched and further impairs mitochondrial function.
in isolated mitochondria from affected brain regions of people with AD. A recent study confirms that APP overexpression in neurons causes its presence in mitochondria-associated membranes. We, for the first time to our knowledge, demonstrate an accumulation of APP in mitochondria-associated membranes. We, for the first time to our knowledge, demonstrate an accumulation of APP in mitochondria-associated membranes.

The induced mitochondrial dysfunction by full-length APP relies on its translocation from the conventional secretory pathway to mis-targeting into mitochondria. Our data suggest a mis-interpretation of the signal sequence of APP as a mitochondrial pre-sequence. This raises several unresolved questions, particularly about the mechanism(s) driving translocation of APP into mitochondrial fractions. At the level of the targeting sequence, previous work suggests that three positively charged residues near the amino-terminal region are responsible for its mitochondrial targeting in neuronal cells. We will examine whether these residues also mediate mis-targeting of APP into mitochondria in white adipocytes in our future efforts. Post-translational modifications are also potential mediators for protein mis-targeting, as indicated by phosphorylation of P4502B1, an ER-targeted component, promoting its translocation into mitochondria. Further study will explore the role of APP in mis-localization from ER to mitochondria.

In summary, we highlight a modulating role of APP in adipocyte mitochondrial function, lipolysis and hypertrophy in the context of obesity. We believe this role of APP as a mediator for adipocyte mitochondrial dysfunction could expand the role of APP as a target for neurodegenerative disease into obesity and associated morbidities.
The mouse model with inducible APP knockout in adipocytes was generated by crossing Adipo-rTA and TRE-Cre mice (B6.Cg-Tg(tetO-cre)1Jaw/J), no. 006234, Jackson Laboratory) together with AppLysm mice (provided by H. Zheng at Baylor College of Medicine), in which the deletion of the App gene was controlled by feeding the mice Dox.

Male C57BL/6J wild-type mice (no. 006664) and ob/ob mice (B6.Cg-Lepr+/−, no. 000632) were purchased from Jackson Laboratory.

HEK293T-rTA cell line. A stable HEK293T-rTA cell line was generated in our laboratory, following previously reported protocols, from a selection from embryonic kidney epithelial HEK293T cells (American Type Culture Collection (ATCC) no. CRL-3216) transfected with cytomegalovirus (CMV) enhancer/promoter-driven CMV-rTA. HEK293T-rTA cells were cultured in DMEM high-glucose (no. 11965-092, Gibco, ThermoFisher Scientific) containing 10% FBS (100-001-BE, Millipore Sigma) and penicillin–streptomycin (50-001-CI, Corning), which was for in vitro Dox-inducible targeted gene expression.

Adipocytes differentiated from stromal vascular fractions. SVFs were isolated from mouse sWAT and cultured in DMEM/F12 medium containing glutamax (no. 10565-018, Gibco, ThermoFisher Scientific) supplemented with 10% FBS, penicillin–streptomycin and gentamicin (no. 15750060, Gibco, ThermoFisher Scientific) in a humid incubator with 10% CO2 at 37 °C. After SVF cells had reached over 95% confluency, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 1 μM dexamethasone, 5 μg ml−1 insulin and 1 μM rosiglitazone were added into the culture medium to initiate in vitro differentiation. After 48 h of incubation, medium containing only 5 μg ml−1 insulin was replaced to maintain the differentiation of SVF-derived adipocytes.

Antibodies and reagents. Anti-APP antibody (no. 101336) for western blotting and immunofluorescence staining was purchased from GeneTex. Antibodies against β-actin (A3854) and amyloid J–1–40/42 (AR5076) as well as amyloid β protein fragment 1–42 (A9810) were from Millipore Sigma. Anti-COXIV antibody (ab14744), anti-PDH-E2 (ab10332) antibody and total OXPHOS rodent WB antibody cocktail (ab10413) were purchased from Abcam. Anti-TIM23 (sc-514463), anti-TOM40 (sc-365467), anti-COXSA (sc-376907) and anti-VDAC (sc-98708) antibodies were obtained from Santa Cruz Biotechnology, Perilipin-1 antibody (20R-PP004) was obtained from Fitzgerald. Primary antibodies against β-tubulin (no. 62698S), calreticulin (no. 12238T), phosphorylated HSL (p-HSL, Ser660, no. 4126S), total HSL (no. 4107), cytochrome C (no. 4272), p-Akt (Ser 473, no. 2808), Akt (Ser 473, no. 9271S) and total Akt (no. 2920S) came from Cell Signaling Technology. Unless specifically indicated, all other reagents were obtained from Millipore Sigma.

Body-composition measurements. For the human study, body fat mass and fat-free mass were determined by using dual-energy X-ray absorptiometry, and intrahepatic triglyceride content was determined by using magnetic resonance imaging.

The precise measurements of mouse whole-body compositions including total body fat and lean mass were performed through the Bruker Minispec mq10 system (Bruker).

Coimmunoprecipitation assay. The assay was performed as previously described. Briefly, isolated mitochondria from sWAT were lysed using Pierce IP lysis buffer (no. 87787, ThermoFisher Scientific) containing protease inhibitor cocktail (CalBioChem). Following an overnight incubation with anti-APP antibody or IgG (negative control) at 4 °C, the lysates were mixed with Protein G Sepharose 4 Fast Flow (no. 17-0618-01, GE Healthcare Life Sciences) at room temperature for 1 h to capture immune complexes. After three sequential washes with Pierce IP lysis buffer, samples were eluted, resuspended in 2× loading buffer and subjected to immunoblotting with the indicated antibodies.

Histological analysis and adipocyte size quantification. Mouse adipose tissues and livers were paraffin embedded, and were cut in 4-μm sections and stained with hematoxylin and eosin (H&E) as well as Masson’s Trichrome C, performed by the University of Texas Southwestern Medical Center Histology Core. Images (x400 or x200 magnification) were recorded by the FSX100 Inverted Microscope (Olympus), and representative histological images are shown.

For adipocyte size quantification, brightfield H&E staining images were taken through a Keyence BZ-X710 microscope (Keyence). Adipocyte size analysis was conducted according to previously validated protocols. Incorporated Keyence BZ-X Analyzer software was utilized for analysing and calculating the area for each adipocyte. At least 60 adipocytes were quantified for each individual mouse.

**Methods**

**Study subjects.** Twenty-four men and women with obesity participated in this study (Supplementary Table 1). All subjects completed a screening history and physical examination, standard blood tests and an OGTT. No subject had diabetes or other serious illnesses, was taking medications that could interfere with insulin action, consumed excessive alcohol (>14 drinks per week for women; >21 drinks per week for men) or smoked tobacco products. Written informed consent was obtained from all subjects before their participation in this study, which was approved by the Institutional Review Board of Washington University School of Medicine. The trial is registered at ClinicalTrials.gov website (NCT02706626).

**Animal models.** All procedures on animals have been approved by the Institutional Animal Care and Use Committee (IACUC) of University of Texas Southwestern Medical Center (APN no. 2015-101207). All experiments were conducted using littermate-controlled male mice and were started when mice were aged 8–10 weeks, except that SVFs were isolated from mice aged 4–6 weeks. All experimental animals were maintained on a C57BL/6 background and housed in a barrier-specific pathogen-free animal facility with 12-h dark–light cycle, and had free access to water and food (chow diet or special diet). Standard chow diet (no. 5058, LabDiet) was regularly fed to the mice. For HFD-only studies, a regular HFD with 60% calories from fat (no. D12492, Research Diets) was fed to the mice. During HFD-challenge experiments together with Dox induction, mice were fed a HFD with 60% calories from fat (no. 5058, LabDiet) was regularly fed to the mice. For HFD-only studies, a regular HFD with 60% calories from fat (no. D12492, Research Diets) was fed to the mice. During HFD-challenge experiments together with Dox induction, mice were fed a HFD with 60% calories from fat (no. D12492, Research Diets) was fed to the mice. During HFD-challenge experiments together with Dox induction, mice were fed a HFD with 60% calories from fat (no. D12492, Research Diets) was fed to the mice.

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**Hyperinsulinemic–euglycemic clamp procedure and adipose-tissue biopsies.** The hyperinsulinemic–euglycemic clamp procedure and AT biopsy were performed in people with obesity, as previously described. Subjects were admitted to the Clinical and Translational Research Unit at Washington University School of Medicine in the evening before the procedure. At 19:00, subjects were served a standard meal, and then they fasted until study completion the next day. At 6:00 the following morning, one catheter was inserted into a forearm vein to infuse

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**Fig. 8** | A modulating role of APP in adipocyte mitochondrial function, lipolysis and hypertrophy in the context of obesity. HFD challenge induces white adipocyte APP overexpression and subsequent mis-targeting into mitochondria, leading to impaired mitochondrial functions. The mitochondrial dysfunction further decreases catecholamine-induced lipolysis, resulting in rapid hypertrophy in adipocytes, followed by a significant obese phenotype and systemic insulin resistance. Adipocyte-specific overexpression of APP recapitu-lates and further accelerates the AT dysfunction and obesity problems, while the specific elimination of App in white adipocytes protects mice from diet-induced metabolic disorders.---
stable isotopically labelled glucose tracer, dextrose and insulin, and a second catheter was inserted into a radial artery in the contralateral wrist to obtain blood samples. At 7:00, a primed (22.5 μmol kg⁻¹), continuous (0.25 μmol kg⁻¹ min⁻¹) infusion of [6,6-2H₂]glucose (Cambridge Isotope Laboratories) began. During the 2-h clamping, a bolus (13 μCi) was injected and followed by a 50-μCi min⁻¹ infusion of [6,6-2H₂]glucose to help to ensure a constant glucose tracer-to-tracee ratio (TTR). Blood samples were collected before beginning the tracer infusion to determine background plasma glucose TTR, and every 10 min during the final 30 min of the basal period and insulin infusion to determine glucose and insulin concentrations and glucose kinetics. Tissue biopsies were obtained during the basal stage of the clamp procedure at around 7:00. After anesthetizing the skin and underlying tissues by percutaneous injection of lidocaine, a small incision in the skin was made, and periumbilical abdominal subcutaneous adipose tissue was aspirated through a 4-mm liposuction cannula connected to a 30-cc syringe. Tissue samples were immediately rinsed with ice-cold saline and frozen in liquid nitrogen before being stored at −80 °C for subsequent RNA extraction.

Hyperinsulinemic–euglycemic clamp experiments and uptake assays of radiolabeled 2-DG ([14C]2-DG, NET326A010MC, PerkinElmer) were performed on conscious unrestrained mice by the Metabolic Phenotyping Core as previously described. Briefly, jugular implantation surgeries were performed on mice 4 d before clamps. On the clamping day, food was removed at 8:00 for a 4-h fast. The primed 3-[14C]glucose (cat. no. NET331C001MC, PerkinElmer) was infused at 0.05 µCi min⁻¹ for 90 min before clamping. During the 2-h clamping, continuous insulin infusion (4 mU per kg per min) and 3-[3H]d-glucose infusion were maintained. Variable infusion of 50% glucose was performed during insulin infusion was maintained by variable-rate infusion of 20% dextrose, saline injection. After 25 min, mice were euthanized, and targeted tissues were plated onto collagen-coated dishes and cultured in 10% CO₂ at 37 °C and 40-μl cell strainers and centrifuged at 600 g, after which the pelleted stromal vascular cells in PBS with 2% FBS. The cells were filtered with 40-μm filter for subsequent RNA extraction.

Mitochondrial DNA copy number. mdDNA copy number was determined as previously described using quantitative real-time PCR of extracted genomic DNA from mouse sWAT. Here, we chose the mitochondrial gene cytochrome c oxidase 3 (mt-CO3) and compared it with nuclear gene NADH dehydrogenase (ubiquinone) flavoprotein 1 (Ndufv1).

Mitochondrial isolation and Percoll-gradient-based purification. Mitochondria and microsomes were isolated and purified from sWAT by using a Percoll gradient fractionation protocol as previously reported. Briefly, tissues were collected and lysed by Dounce homogenization in sucrose isolation buffer (0.25 M sucrose in 10 mM HEPES, pH 7.4), and protease inhibitor cocktails were added. After centrifugation at 10,300 g at 4 °C for 15 min, the total cytoplasm fractions including microsomes (ER, Golgi apparatus and plasma membrane) and the crude mitochondrial fraction were collected. The obtained crude mitochondrial fraction was further purified through a Percoll gradient (40 Percoll and 0.25 M sucrose in 10 mM HEPES, pH 7.4) using a Sorvall Discovery M150 SE (ThermoFisher Scientific) ultracentrifuge, spinning at 288,000 r.p.m. at 4 °C for 40 min. The diffuse mitochondrial band was then transferred to new tubes and centrifuged at 10,000 g for 10 min to pellet the purified mitochondria. After resuspending the mitochondrial pellet, protein concentrations were determined by the bicinchoninic acid (BCA) assay kit (Thermo Scientific Pierce) and subject to immunoblotting or mass-spectrometry-based proteomic analysis performed by the University of Texas Southwestern Proteomics Core. The secretome was analysed and predicted through the Vertebrate Secretome Database (http://genomics.cibigene.edu.au/VerbDx/index.php).

Mitochondrial membrane potential staining. Mitochondrial membrane potential (MMP) was assessed by staining the cells with a commercially available MitoStatus dye tetramethylrhodamine ethyl ester (TMRE, no. 564969) from BD Biosciences. Briefly, sWAT-derived adipocytes, which were then cultured in glass-bottom microwell dishes and were induced for 48 h and then incubated with 200 nM TMRE for 20 min at 37 °C. After a gentle wash, the cells were fixed, and fluorescence images were captured using the Keyence BX-ZX10 microscope.

Mitochondrial protein import assay. The assay of mitochondrial protein import was performed as previously described, with a few modifications. Briefly, sWAT-differentiated adipocytes, which were then cultured in glass-bottom microwell dishes and were induced for 48 h and then incubated with 200 nM TMRE for 20 min at 37 °C. After a gentle wash, the cells were fixed, and fluorescence images were captured using the Keyence BX-ZX10 microscope.

Mitochondrial respiration measurement. Mitochondrial respiration was determined using the Seahorse XF24 Extracellular Flux Analyzer (Agilent) following the manufacturers’ instructions. In brief, a mitochondrial stress test was performed according to a manufacturer-recommended BOFA (basal-oligomycin-FCCP-antimycin A/rotenone) protocol. Ex vivo and in vitro mitochondrial function were measured by utilizing 5–10 mg sWAT fat pads and sWAT-differentiated adipocytes, respectively. For tissues, oligomycin (2 μM), FCCP (8 μM) and antimycin A (10 μM) plus rotenone (3 μM) were added; for cultured
cells, oligomycin (1 μM), FCCP (4 μM) and antimycin A (10 μM) plus rotenone (1 μM) were injected. OCR and extracellular acidification rate were recorded through the Seahorse instrument.

Mitochondrial sub-fractionation. After isolation and percoll-purification of AT mitochondria, pelleted mitochondria were subjected to further sub-fractionation as previously described. In short, 60 mitochondrial pellets (inner membranes surrounding the mitochondrial matrix) were pelleted and the low-speed supernatants were collected. The mitoplast pellet was subject to subtle sonication through a Diagenode Bioruptor (UCD-300, Diagenode). Both post-sonication mitoplasts and the supernatants were further centrifuged at 144,000 × g for 1 h to generate two sets of pellets (the inner-membrane pellet from the mitoplasts and the outer-membrane pellet from the low-speed supernatants) and supernatants (the matrix fraction, from the mitoplasts and the intermembrane space fraction from the low-speed supernatant). The four fractions were stored at −80°C until further analysis through western blotting.

RNA extraction and real-time quantitative PCR. Upon euthanization of mice, depots of adipose tissues including sWAT, eWAT, mesenteric WAT, perirenal WAT and BAT, as well as liver and brain tissues, were collected and quickly frozen in liquid nitrogen for future use. For the total RNA extraction, a hybrid protocol of TRIzol (Invitrogen) reagents and the RNeasy RNA extraction kit (no. 74106, Qiagen) was performed. Briefly, after homogenizing the tissues by using a Tissuemizer (Qiagen), the RNAs were isolated following the protocol according to the RNeasy kit. Then the quality and concentration of the RNA were determined through the NanoDrop spectrophotometer (N1-1000, Thermo Scientific). A total of 1 μg RNA underwent subsequent reverse transcriptional reactions with an iScript cDNA synthesis kit (no. 170-8891, Bio-Rad). CDNAs were diluted and stored, and used in further qPCR quantification of relative gene expression. Briefly, we used the SYBR Green PCR Master Mix (A25742, Life Technologies) to perform the qPCR reactions, and the experiments were conducted on an ABI Prism 7900HT Real-Time PCR System or a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems). All the primer sequences in this study were validated in previous studies and are listed in Supplementary Table 2, and human Gapdh and RPS18 was used as the internal control for relative quantification.

Serum and liver chemistry. Upon euthanization of the mice, whole blood samples were obtained and centrifuged at 6,000 r.p.m. for 15 min to collect the serum. Samples were analysed for glucose, cholesterol, triglycerides and free-fatty-acid (NEFA) measurements through a Vitros 250 Chemistry system (Ortho Clinical Diagnostics). Similarly, after homogenizing –100 mg of frozen liver tissues, the lysates were utilized for tissue cholesterol and triglycerides measurements after extraction.

Systemic metabolic tests. Systemic metabolic tests in this study included body-weight monitoring, OGTT and ITT. For the OGTTs, 5-h fasting was conducted prior to administration of 2.5 g per kg (body weight) glucose to the mice through oral gavage. Blood was collected from tail veins at each time point indicated, and the blood samples were centrifuged at 6,000 r.p.m. for 15 min and stored for further measurements. Glucose concentrations were measured by using an oxidase–peroxidase-based colorimetric assay (Sigma-Aldrich). Insulin levels were measured through a commercially available ELISA kit (no. 80-INSMSU-E01) purchased from Alpco. For the ITTs, mice underwent 5-h fasting before 1 unit insulin per kg (body weight) was administered through intraperitoneal (i.p.) injection. Blood samples were collected at the indicated time points and utilized for glucose measurements.

Transmission electron microscopy and visualization of APP in mitochondria. Briefly, sWAT from different mice was fixed by perfusion with a fixation buffer (0.1 M sodium cacodylate containing 4% paraformaldehyde and 1% glutaraldehyde), and the collected tissue was then transferred to 2.5% glutaraldehyde in 0.1 mM sodium cacodylate buffer and cut into pieces. Then the tissue pieces were sent to the University of Texas Southwestern Medical Center for transmission electron microscopy sample processing performed by the University of Texas Southwestern Electron Microscopy Core facility.

Western blot analysis. After frozen tissue samples were homogenized, they were lysed in RIPA buffer (1% Triton-X100, 50 mmol l−1 Tris-HCl (pH 8.0), 0.25 mol l−1 NaCl, 5 mmol l−1 EDTA) containing phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail (Calbiochem). Following centrifugation at 12,000 × g for 15 min at 4°C, the supernatants were collected, and protein concentrations were measured by the BCA kit. For immunoblotting, protein samples (20–60 μg) or serum (0.1 μl diluted in 10 μl Tris-buffered saline Tween (TBS-T, 20 mmol l−1 Tris, 137 mmol l−1 NaCl, pH 7.6) were loaded and separated by 4–12% Super polyacrylamide gel electrophoresis (SDS-PAGE). Upon completion of the electrophoresis, the proteins were transferred to a nitrocellulose membrane. Non-specific binding sites were blocked by preincubating the membrane in 5% BSA in TBS-T. The membranes were then incubated overnight at 4°C with specific primary antibodies: anti-APP (1:1,000), anti-COXIV (1:1,000), anti-β-tubulin (1:1,000), anti-calreticulin (1:1,000), anti-β-actin (1:5,000), anti-adiponectin (1:2,000), anti-phHSL (1:1,000), anti-HSL (1:1,000), anti-amyloid β 1-40/42 (1:1,000), anti-OXPHOS rodent WB antibody cocktail (1:1,000), anti-PDH-E2 (1:1,000), anti-TIM23 (1:500), anti-cytochrome C (1:1,000), anti-VDAC (1:2,000), anti-TOM40 (1:400), anti-COX5A (1:500), anti-p-Akt (1:1,000) and anti-Akt (1:1,000). After washing, fluorescent-conjugated secondary antibodies (Invitrogen) were added and incubated at room temperature for 1 h. Then after three washes, the western blot bands were scanned by the LI-COR Odyssey Imager, and the band intensity was analysed through the LI-COR Odyssey Imaging software.

Statistical analysis. All data are presented as means ± s.e.m. of biologically independent samples, except that data are presented as mean ± s.d. in Supplementary Table 1. We utilized GraphPad Prism 7.04 (GraphPad Software) to perform the statistical analyses. For computing correlation coefficients, Pearson correlation calculations were performed. For comparisons between two independent groups, a two-tailed Student’s t-test was used. Differences between two groups over time were determined by a two-way ANOVA followed by a Tukey post-test was used. Differences between two groups over time were determined by a two-way ANOVA followed by a Tukey post-test to compare replicate means in each time point. P < 0.05 was considered statistically significant, and exact P values are labelled in figures.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data that support the findings of this study are included in the figures, extended data figures and Supplementary Information. Proteomics raw data has been deposited to the Mass Spectrometry Interactive Virtual Environment (MassIVE) database (MSV000844919).

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**Author contributions**

Conceptualization, Y.A.A., I.W.A. and P.E.S.; methodology, Y.A.A. and K.S.; formal analysis, Y.A.A.; investigation, Y.A.A., C.C., S.F., E.Z., M.S., J.-B.F., Z.Z., I.S. and C.M.K.; resources, J.Y. and S.K.; original draft writing, Y.A.A.; manuscript review and editing, C.C., I.W.A., S.K., C.M.K. and P.E.S.; supervision, P.E.S.; funding acquisition, P.E.S.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | APP is increased in WAT in obesity and adipocyte-specific APP overexpressing mice are more sensitive to diet induced obesity (related to Fig. 1a,h). a–f, The correlation between APP mRNA levels in sWAT from people with obesity with body mass (a), BMI (b), subcutaneous AT volume (c), triglycerides (d), fasting insulin (e) and HDL-cholesterol (f) levels. n = 23 (a–d) or 24 (e,f) subjects. g, App transcription in the brain in acute HFD-challenged wild-type mice for 0, 1, 2, 5, 7 and 14 d. n = 7 for Day 0 group; n = 5 for Day 1, 2, 5, 7 and 14 groups. Data are shown as mean ± s.e.m. of biologically independent samples. Pearson correlation analysis for correlation coefficient (r) and two-tailed P value (a–f); one-way ANOVA followed by a Tukey post-test, and non-significance was found (g).
Extended Data Fig. 2 | Adipocyte-specific APP-overexpressing mice are more sensitive to diet-induced obesity (related to Fig. 2c,d,g). a, Representative western blotting image for APP protein levels in brain samples from control and APP transgenic mice under HFD/Dox feeding. n = 3 mice per group. b, Western blotting for APP in mitochondrial and post-mitochondrial fractions from sWAT in control and APP-overexpressing mice fed with Dox for 1 week. COXIV, mitochondrial marker; β-tubulin, cytoplasmic marker. n = 2 mice per group. Representative image chosen from three independent experiments in a and b. c, Immunofluorescence staining for APP (red), TIM23 (green) and DAPI (blue) for nuclear labelling in HEK293T cells transfected with empty vector (left) or the mito-APP construct (right). Orange colours indicate the merge between APP and TIM23. Scale bars, 20 μm. The staining experiments have been replicated three times. d, Insulin levels measured in serum samples obtained from OGTT experiments. n = 8 mice per group. Data are shown as mean ± s.e.m. of biologically independent samples. Two-way ANOVA followed by a Tukey post-test (d).
Extended Data Fig. 3 | APP overexpression leads to adipocyte hypertrophy by impairing stimulated lipolysis (related to Fig. 4a–j). a, Representative images for H&E staining in sWAT sections from control mice (APP- ) at different time points following induction with Dox (600 mg per kg (diet weight)), chosen from two independent experiments; scale bars, 161 μm. b, Representative H&E staining images in eWAT sections from APP transgenic mice (APP+ ) at different time points following induction with Dox (600 mg per kg (diet weight)), chosen from two independent experiments; scale bars, 100 μm. c–e, Lipogenesis-related gene transcriptions in sWAT of APP transgenic mice at different time points of Dox 600 feeding (600 mg per kg (diet weight)): Dgat2 (c), Fasn (d), Scd1 (e) and Srebp1 (f); n = 3 mice per time point. Data are shown as mean ± s.e.m. of biologically independent samples. One-way ANOVA followed by a Tukey post-test (c–f) and no statistical significance was found.
Extended Data Fig. 4 | APP impairs adipocyte mitochondrial function owing to defective mitochondrial protein import (related to Fig. 5a,b,f).

**a.** In vitro mitochondrial respiration (OCR) in sWAT SVF differentiated adipocytes from control (APP–) and APP transgenic (APP+) mice. Cells are pre-incubated for 5 μg ml⁻¹ Dox for 48 h to induce APP overexpression. *n* = 5 per group.

**b.** Relative in vitro ATP production changes from SVF-differentiated adipocytes of APP transgenic mice compared with control mice. Different dosages of Dox were applied to cells. *n* = 8 per dosage.

**c.** Combined light and fluorescence microscopy images displaying mitochondrial membrane potential (MMP) through TMRE staining (Red) from SVF-differentiated adipocytes of control and APP transgenic mice. DMSO serves as the negative control, and FCCP is a positive control to collapse MMP. Images were chosen from three independent experiments and are representative of at least 12 fields for each group. Scale bars, 50 μm.

**d–g.** Quantification for indirect calorimetry measurements in control and APP transgenic mice in light and dark cycles: oxygen consumption (VO₂) (**d**); CO₂ production (VCO₂) (**e**); RER (**f**) and calculated energy expenditure (**g**). *n* = 6 mice per group.

**h.** Western blotting for Aβ-40/42 in mitochondrial and cytoplasmic fractions from sWAT in control and APP-overexpressing mice. Aβ 1-42 protein has been loaded separately as a positive control. *n* = 2 mice per group. A representative image was chosen from three independent experiments.

**i.** Western blotting for mitochondrial complex components using oxidative phosphorylation antibody cocktail in mitochondrial and cytoplasmic fractions from sWAT in control and APP-overexpressing mice. **k.** Western blotting for COX5A in sWAT in control and APP-overexpressing mice. *n* = 3 mice per group. A representative image was chosen from three independent experiments.

Data are shown as mean ± s.e.m. of biologically independent samples. Two-way ANOVA (**a**); one-way ANOVA followed by a Tukey post-test (**b**); Two-tailed Student’s t-test (**d–g,j**).
Extended Data Fig. 5 | Dysregulation of Srp54c is responsible for mistargeting of APP into adipocyte mitochondria (related to Fig. 5). a,b, Srp subunit gene mRNA levels in floating adipocytes in 30-week-HFD- and chow-fed mice (a, left, sWAT; right, eWAT; n = 3 mice per group) and Srp54c mRNA levels from sWAT in acute-HFD-challenged wild-type mice for 0, 1, 2, 5, 7 and 14 d (b); n = 7 mice in Day 0 group and n = 5 mice in Day 1, 2, 5, 7 and 14 groups. c, The correlation between Srp54c mRNA levels in sWAT from HFD-challenged wild-type mice with App mRNA expression; n = 32.
d, Schematic illustration of the adipocyte-specific, Dox-inducible Srp54c transgenic mouse model. e, Validation of Srp54c overexpression in WAT of transgenic mice fed with 1-week Dox (600 mg per kg (diet weight)) diet by detecting Srp54c mRNA levels in different tissues from control (Control) and Srp54c overexpressing (Srp54c Tg) mice (n = 4 mice per group). f, Representative western blotting image (left) for APP in purified mitochondrial, post-mito and whole tissue lysate from sWAT in 1-week Dox-fed mice, and its quantification (right panel). n = 3 mice per group. Images are chosen from three independent experiments. g, Representative autoradiography image (left) and statistics (right) of pOTC import assessed in isolated mitochondria (incubation for 30 min) from sWAT of 1-week Dox-fed control or Srp54c Tg mice; 25% of [35S]pOTC was added to each reaction is loaded as input. n = 4 mice per group.
h–i, Upon 3-week Dox feeding, both control and Srp54c Tg mice are subject to metabolic analysis, including body weight monitoring (h) and OGTT assays (i); n = 4 mice per group. j, Proteomics analysis performed in purified mitochondria from WAT of control and Srp54c Tg mice. Left, percentage of secretome proteins among uniquely detected proteins in Srp54c Tg mitochondria; right, heat map depicting enrichment of identified proteins belonging to the secretome (indicates a polypeptide containing a well-defined ER signalling sequence). For all statistical graphs, numeric data are presented as mean ± s.e.m. of biologically independent samples. Two-tailed Student’s t-test (a–g); one-way ANOVA followed by a Tukey post-test (b); Pearson correlation analysis for correlation coefficient (r) and two-tailed P value (c); two-way ANOVA followed by a Tukey post-test (h,i).
Extended Data Fig. 6 | Halting APP overexpression reverses the obese phenotypes (related to Fig. 6). a–d, APP-overexpressing mice (APP+) were fed with HFD/Dox diets for 8 weeks and were divided into two groups, one group continuously on HFD/Dox feeding (Keep Dox) and the other group fed with HFD without Dox (Withdraw Dox). Two groups of mice are subject to the following metabolic analyses (n = 6 mice per group). a, Body weight for 12 weeks. b, Ex vivo mitochondrial respiration (OCR) in sWAT fat pads from both groups, n = 10 tissues per group. c, d, Glucose levels at different time points from OGTT (c) and ITT experiments (d). e,f,h, Control mice (APP−) were fed with HFD/Dox diets for 8 weeks, and the cohorts were divided into two groups, one group continuously on HFD/Dox feeding and the other group fed with HFD without Dox. Two groups of mice were subjected to the following metabolic analyses. e, Body weights for the 12-week exposure (n = 6 mice per group). f, Ex vivo mitochondrial respiration (OCR) in sWAT fat pads from both groups (n = 10 tissues per group). h, Glucose levels at different time points from (n = 6 mice per group) OGTT experiments. g, Combined high-resolution respirometry measured in isolated mitochondria from four groups including control or APP transgenic mice kept with or without Dox feeding, n = 5 mice per group. i, j, AT inflammatory and liver steatosis phenotypes in control or APP transgenic mice kept with or without Dox feeding. i, Representative H&E staining images for sWAT. j, Representative H&E staining images of liver tissues from four groups. Images are chosen from three independent experiments. For all the statistics: data are presented as mean ± s.e.m. of biologically independent samples. Two-way ANOVA followed by a Tukey post-test (a–h), and no statistical significance was found in e, f and h.
Extended Data Fig. 7 | App AKO protects mice from obesity with enhanced adipocyte mitochondrial function (related to Fig. 6e–h). a, b, Experiments were in 12-week HFD/Dox feeding control or App AKO mice. a, Insulin levels measured in serum samples obtained from OGTT experiments. b, Glucose levels at difference time points during ITT assays. n = 8 mice per group. c–g, For insulin-sensitivity measurement, three groups of mice, including control, APP adipocyte-specific transgenic (App Tg) and App adipocyte-specific knockout (App AKO) mice were subjected to hyperinsulinemic–euglycemic clamp studies. c, Body weight. d, Clamped glucose levels. e, GIR. f, Basal hepatic glucose production. g, 2-DG uptake in different metabolic tissues. For c–f, n = 7 mice in control and App Tg groups, n = 6 mice in App AKO group. For g, n = 6 mice per group. h, i, Representative immunoblot image of p-Akt (Ser 473) and total Akt expression in different metabolic tissues from both control and APP-overexpressing mice (h) or both control and App AKO mice (i) after saline or insulin injection (i.v.) for 5 min. For the western blot image, n = 3 mice per group, and the representative images are chosen from three independent experiments. Dare shown as mean ± s.e.m. of biologically independent samples. Two-way ANOVA followed by a Tukey post-test (a,b); one-way ANOVA followed by a Tukey post-test (c–g).
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Software and code

Policy information about availability of computer code

Data collection

Keyence BZ-X Analyzer software (version 1.3.0.3, Keyence, Itasca, IL, USA) was utilized for analyzing and calculating the area for each adipocyte. The radioactive OTC on the membrane were visualized through the Bio-Rad Pharos FX Plus Molar Imager and then quantified through the Bio-Rad Quantity One 1-D analysis software (version 4.6.6, Bio-Rad Laboratories, Inc., Hercules, CA, USA). Mitochondrial respiration data were viewed and analyzed through the Wave software (version 2.6.0, Agilent, Santa Clara, CA, USA). Ct values for qPCR data were calculated by QuantStudio Real-Time PCR software (version 1.2, Applied Biosystems, Foster City, CA, USA). Western blot band intensity was analyzed through the LI-COR Odyssey Imager software (version 3.0, LI-COR, Lincoln, NE, USA).

Data analysis

We utilized GraphPad Prism 7.04 (GraphPad Software, Inc., La Jolla, CA, USA) to perform the statistical analyses.

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- **Sample size**: We determined the sample size based on previous published papers containing similar procedures and pilot experiments showing trends of effects. All the sample size information is referred to in the figure legends. The current sample size is considered to be sufficient since we saw statistical differences and acceptable variations in replicated independent experiments.

- **Data exclusions**: In Extended Data Figures 7c-g, mice were excluded due to incomplete procedure during the hyperinsulinemic-euglycemic clamp experiments, following the exclusion criteria described in the 2012 handbook of Glucose Clamping in the Conscious Mouse from Vanderbilt Mouse Metabolic Phenotyping Center.

- **Replication**: We defined each mouse or each sample in different groups as biological replicate; and we defined repeating independent mouse cohorts (for metabolic studies) or performing independent biological experiments (such as qPCR, western blot, etc.) as technical replication. For the information about biological replication, please refer to sample size. For technical replication, at least three independent cohorts and three biological experiments were conducted. We confirm successful replication for our reported data.

- **Randomization**: Mice at the same age with different genotypes were randomly allocated to groups (such as Chow diet vs. HFD groups, or different time-points).

- **Blinding**: In this study, no blinding has been performed, since the experimental design requires the investigators to know genotyping information for each mouse. However, based on the randomization in all assays, although without blinding performance, we can keep completely unbiased ways to collect and analyze data.

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| ☐ Palaeontology                 | ☐ MRI-based neuroimaging |
| ☐ Animals and other organisms   |         |
| ☐ Human research participants   |         |
| ☐ Clinical data                 |         |

Antibodies

**Antibodies used**

- Anti APP antibody (#101336, C2C3, Lot No. 39694) for western blotting (1:1000) and immunofluorescence staining (1:200) was purchased from GenTex (Irvine, CA, USA). Antibodies against β-actin (A3854, AC-15, Lot No. 084A4770V, 1:5000) and amyloid β 1:40/42 (AB5016, Lot No. 30633860, 1:10000) as well as amyloid β protein fragment 1-42 (A810) were from Millipore Sigma (St. Louis, MO, USA). Anti COXIV antibody (ab14744, 2008C12, Lot No. GRB242129, 1:1000), P0H-E2 (ab110332, 150369C11, Lot No. BAASH, 1:1000) antibody, and total OXPHOS rodent WB antibody cocktail (ab10413, Lot No. P5685, 1:1000) were purchased from Abcam (Cambridge, MA, USA). Anti-TIM23 (sc-54463, H-8, Lot No. 80218, 1:500 for WB and IF), anti-TOM40 (sc-365467, D-2, Lot No. A2208, 1:400), anti-VDAC (sc-98708, FL-283, Lot No. I2910, 1:200), and anti-COX5A (sc-576907, A-5, Lot No. F1109, 1:500) antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Perilipin-1 antibody (2D-PP004, Lot No. F118120501, 1:500 for IF) was obtained from Fitzgerald Inc. (Acton, MA, USA). Primary antibodies against β-tubulin (#628985, D211W, Lot No. 1, 1:1000), calreticulin (#12328T, D5E6, Lot No. 4, 1:1000), phosphorylated HSL (pHSL Ser680), #41265, Lot No. 3, 1:1000, total HSL (#4107, Lot No. 3, 1:1000), cytochrome C (#2472, Lot No. 1, 1:1000), phosphorylated Akt (pAkt, Ser473, #92715, D9E, Lot No. 24, 1:1000) and total Akt (#29205, 04D4, Lot No. 8, 1:1000) came from Cell Signaling Technology Inc. (Beverly, MA, USA).

**Validation**

According to the manufacturer’s website, all the above commercial antibodies have been validated for the applications of western blot or immunofluorescence staining in the mouse species. In addition, β-actin (A3854), COXIV antibody (ab14744), total
Eukaryotic cell lines

Policy information about: cell lines

Cell line source(s)  Embryonic kidney epithelial HEK293T cells were obtained from ATCC (# CRL-3216).

Authentication  HEK293T cells were originally purchased from ATCC and verified for its competence to replicate vectors carrying the SV40 region of replication.

Mycoplasma contamination  HK2931 cells were tested negative for mycoplasma contamination during our experiments.

Commonly misidentified lines (See ITAG registry)  No commonly misidentified cell lines were used in the study.

Animals and other organisms

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Laboratory animals  Male C57BL/6J mice (No. 000664) and Ob/Ob mice (86 Cg-Lepob/J, No. 000643) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All the genetic mouse models described in the study (TRC-mito-APP, Adipo-rTAs, TRE-SRP54c, TRE-Cre, ApoI/lox/lox) are maintained on a C57BL/6J background, and in all the reported data, male mice were employed. For SVF isolation, mice of 4-6 weeks old were utilized. All the other animal studies were started at the age of 8-10 weeks old.

Wild animals  No wild animals were employed in this study. All experimental animals were housed in a barrier specific pathogen free animal facility with 12-hour dark-light cycle with free access to water and food.

Field-collected samples  No field-collected samples were utilized in this study.

Ethics oversight  All performed procedures on animals have been approved by the Institutional Animal Care and Use Committee (IACUC) of UT Southwestern Medical Center at Dallas (AP# 2015-101207).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about: studies involving human research participants

Population characteristics  Twenty-four men and women with obesity participated in this study (Age [yr]: 38 ± 6; Sex (M/F): 3/21; Body mass index (kg/m2): 38.6 ± 5.0; more detailed information in Supplementary Table 1).

Recruitment  Subjects were patients admitted to the Clinical and Translational Research Unit at Washington University School of Medicine and voluntarily participated in this study. All subjects completed a screening history and physical examination, standard blood tests and an oral glucose tolerance test. No subject had diabetes or other serious illnesses, were taking medications that could interfere with insulin action, consumed excessive alcohol (>14 drinks/week for women and >21 drinks/week for men), or smoked tobacco products. We didn’t identify any self-selection or other biases to impact the results in this paper.
Ethics oversight

Written informed consent was obtained from all subjects before their participation in this study, which was approved by the Institutional Review Board of Washington University School of Medicine in St. Louis, MO. The trial is registered at ClinicalTrials.gov website [NCT02706262].

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