Dietary excess regulates absorption and surface of gut epithelium through intestinal PPARα

Intestinal surface changes in size and function, but what propels these alterations and what are their metabolic consequences is unknown. Here we report that the food amount is a positive determinant of the gut surface area contributing to an increased absorptive function, reversible by reducing daily food. While several upregulated intestinal energetic pathways are dispensable, the intestinal PPARα is instead necessary for the genetic and environment overeating–induced increase of the gut absorptive capacity. In presence of dietary lipids, intestinal PPARα knock-out or its pharmacological antagonism suppress intestinal crypt expansion and shorten villi in mice and in human intestinal biopsies, diminishing the post-prandial triglyceride transport and nutrient uptake. Intestinal PPARα ablation limits systemic lipid absorption and restricts lipid droplet expansion and PLIN2 levels, critical for droplet formation. This improves the lipid metabolism, and reduces body adiposity and liver steatosis, suggesting an alternative target for treating obesity.
obesity is caused by an energetic disbalance following chronic excess of caloric intake over energy expenditure. Which of the two is more decisive in the modern setting has long been debated. Almost all caloric uptake takes place in intestine. However, understanding the relationship between overeating, gut absorption, and obesity is in its infancy. We and others have shown that environmental or nutritional stimuli induce changes in gut morphology, which may alter efficiency of caloric uptake, contributing to energy homeostasis. While the data on the correlation between the intestinal, villi and microvilli length and the body mass index (BMI) in humans is scarce and contradicting, intestinal hyperplasia is a frequent feature of diabetic hyperglycaemia.

Maintenance and expansion of the gut epithelium depends on the activity of Lgr5 intestinal stem cells (ISCs), which give rise to Paneth cells and rapidly dividing transitory progenitors in intestinal crypts. Along the villi, these cells differentiate into enterocytes, enteroendocrine cells and goblet cells that eventually undergo apoptosis and shedding near the top. Caloric restriction through Paneth cell-mediated, reduced progenitor differentiation and villi length, whereas obese mice have longer villi and larger intestines. High-fat diet (HFD) supports division of intestinal stem cells and tumorigenicity; however, mice fed HFD end up having shorter intestines and villi in contrast, energy-consuming cold exposure shifts intestinal homeostasis towards longer guts and villi, in concert with increased caloric uptake in conditions of increased metabolic demand.

Another metabolic consideration unique to the intestine is that it must distribute nutrients for the rest of the organism while maintaining high metabolic rate for its own needs. As a result, the intestine is a metabolically diversified organ with a complex interplay between processes in the crypt and gut plasticity. ISC metabolism rely on carbon substrates from Paneth cells and their own oxidative metabolism. In enterocytes, glutamate is the preferred substrate for oxidation, and is oxidised in both fed and fasted state (from deaminated glutamine), while sugars and fatty acids are released for use elsewhere and fasted state (from deaminated glutamine), caloric uptake from the intestine.

In this work, we investigate the energy metabolism of the adult gut plasticity triggered by nutritional changes and address the physiological consequences of intestinal metabolic perturbations. We show that intestinal absorptive surface and function are regulated by the amount of consumed food, causing an adaptive increase in the intestinal absorptive capacity in conditions of increased food availability. Using multiple intestine-specific genetic mouse knockouts and human intestinal biopsies, we systematically addressed what metabolic pathways are decisive in controlling the gut plasticity and function. Our work demonstrates that in presence of dietary lipids, PPARa-dependent transcription programs are necessary for enlargement of the intestinal surface through increasing villi length, and for both overall nutrient and triglyceride uptake by enterocytes. Unexpectedly, while PPARα promotes catabolism and fatty acid oxidation in various organs, we found that intestinal PPARα deletion or its pharmacological antagonism leads to concomitant reduction of lipid droplet (LD) amount and size, decreased fatty acid transport, and depletion of perilipin 2 (PLIN2), a critical regulator of LD formation. Our work thus proposes that in presence of dietary lipids, the intestinal LD formation and trafficking regulated by PPARα are important rate-limiting steps in the systemic lipid metabolism, and in the food amount-driven intestinal surface enlargement.

**Results**

**The amount of ingested food dictates the intestinal absorptive surface and capacity.** We first examined intestinal size in genetically obese mice. Both ob/ob and db/db mice spontaneously overeat throughout their lifetime due to missing satiety signal, conferred by the lack of leptin or its receptor, respectively. Compared to WT mice, ob/ob mice had longer and heavier small intestines (Fig. 1a, Supplementary Fig.1a), similar to the increase seen in db/db mice (Supplementary Fig.1b). The perimeter of the jejunum was larger (Fig. 1b), and villi and microvilli longer in ob/ob jejunum (Fig. 1c, d), thereby vastly increasing the absorptive surface at multiple levels. In ob/ob mice, where increased body weight is primarily due to increased fat accumulation, the length of the small intestines correlated with the body weight (Fig. 1e), while being independent of the lean (mass) mass (Fig. 1f). Analysis of faeces by bomb calorimetry revealed increased caloric extraction from ingested food in ob/ob mice (Fig. 1g). Accordingly, increase in absorptive surface of the genetically-induced overeating obese mice correlated with increased body weight and caloric uptake from the intestine.

To determine if intestinal size correlates with food intake, we compared intestinal sizes (Fig. 1h) in ob/ob and in several groups of C57BL/6J (WT) male mice (Charles River France) at 14 weeks of age, which differed in the amount of eaten food (Fig. 1i) due to exposure to a different temperature, food type or caloric restriction. Ob/ob mice ate 50% more standard chow food than WT. However, when the amount of chow given to ob/ob was restricted to the same amount as eaten by WT during six weeks (ob/ob CR), the small intestine of ob/ob decreased in length and width (Fig. 1h, Supplementary Fig.1c). Similarly, WT mice calorically restricted to 60% of ad libitum standard chow, decreased their intestinal length (Fig. 1h). Intestinal length increased in mice where overeating was induced by a 30-day 6°C exposure. This increment was attenuated when food amount was restricted from ad libitum to the minimal necessary levels for 6°C exposure. This increment was attenuated when food amount was restricted from ad libitum to the minimal necessary levels for 6°C exposure. To further address if the amount of eaten food per se, rather than obesity drives the intestinal enlargement, we assessed the gut surface following multiple feeding regimens with food of different caloric densities and food intakes. Mice eat smaller quantities of high-fat diet than of standard chow due to higher energy density of HFD, but that still translates to more calories taken in (Fig. 1i, j). Depending on the exact type of high-fat diet, intestinal length was unchanged or shortened compared to normal chow-fed mice. The calorically most dense high-fat, high-sucrose diet (HF-HS), and fibre-poor, butter-derived HFD shortened the gut (Fig. 1i, Supplementary Fig.1d, Supplementary Table 2). In an interesting contrast to HFD, the energetically depleted food (lower caloric density per gram) demanded overeating and led to gut extension (Fig. 1h, i).

A comparison of these conditions revealed a correlation between average intestinal length and the weight of consumed food.
(Fig. 1k). We found a positive relationship between food intake and average villus length in jejunum (Fig. 1l–n). Food intake and microvilli length of the jejunal brush border, strongly matched in WT HFD, WT chow, and ob/ob chow mice. (Fig. 1o). Consistent with the recent report, HF-HS mice had slightly increased villi length likely due to the fructose present in this diet. The correlation between food amount and intestinal plasticity was further tested against variations that could arise from different food mixtures and mice suppliers. Three chow mixtures from different suppliers caused similar food intake and intestinal length (Supplementary Fig.1e, g), though interestingly, a protein-rich, soya and fish-derived diet had increased starting villi length compared to other two vegetable-based foods (Supplementary Fig.1f). C57BL6/J from Janvier had longer intestines on chow diet compared to mice obtained through Charles River, and HFD produced stronger surface decrease in these mice (Supplementary Fig.1h, i). This suggests that factors such as macronutrient composition or microbiota can influence starting intestinal surface, which is then strongly modified by the food intake. Taken together, these data show that while keeping constant genetic background, age, sex, SPF housing, the intestinal absorptive area positively correlates with the amount of eaten food in respective mice, independently of the caloric intake from that food.

Multiple energetic pathways are upregulated, but dispensable for the increased intestinal surface and function in vivo. Next, we systematically analysed the upregulation of metabolic pathways in elongated guts. Pathway enrichment analyses of transcriptomes from jejunum of cold-exposed mice revealed that energy conversion pathways, such as glutamate and glucose metabolism, as well as
oxidative phosphorylation were among the top upregulated hits. Intestinal PPARα is necessary for villi elongation. In search of the mechanisms for the altered intestinal function, we next measured soluble metabolites in jejunum samples of cold-exposed mice. The top enriched metabolic pathways as determined by metabolomics were biosynthesis and metabolism of hydroxyecosatetraenoic acids (HETE) (Fig. 2c), which are derivatives of arachidonic acid. HETEs activate PPARs, including PPARα. Branched-chain aminoacids and glycolytic and gluconeogenic metabolites were also high after cold exposure. Eicosapentaenoic acid (EPA), another eicosanoid that binds and activates PPARs, was elevated in jejunum of cold-exposed mice (Fig. 2d). The critical regulator of the tricarboxylic acid (TCA) cycle, Pdk4, which favours β-oxidation by negatively regulating the enzyme that channels glycolytic carbons into the TCA cycle, was strongly upregulated in cold and ob/ob (Fig. 2b). The two main PPAR isoforms expressed in intestines are PPARα and PPARδ, while PPARγ is ten times less abundant (Fig. 2e). Of these, Ppara expression is the most abundant in jejunum and the only one that was upregulated by cold exposure and in ob/ob mice. Expression of transcriptional regulator Prdm16 and PPARα co-activator Rxr were not changed, while the PPARα target Nr1h4 (coding for FXR) that regulates fatty acid oxidation was induced in ob/ob mice. We used the previously described Villin-Cre x Ppara lox/lox mice (Ppara I-KO, Supplementary Fig. 6a-f) to study the role of intestinal fatty acid oxidation in gut expansion. We first exposed mice to 6°C for 30 days to provoke gut expansion (Fig. 2f–l, room temperature controls in Supplementary Fig. 6g–l). The Ppara I-KO mice exhibited better oral glucose tolerance, particularly at the early absorption stage (Fig. 2f), which was not the case for intraperitoneal injection (Fig. 2g), and not due to different insulin sensitivity (Fig. 2h). Ppara I-KO mice had lower fat mass compared to their controls after 30 days of cold exposure (Fig. 2i), owing to a reduction in both subcutaneous and visceral fat depots. The gut length increased in both Ppara I-KO and control mice upon cold exposure compared to room temperature (Fig. 2), though I-KO trended toward a smaller perimeter (Fig. 2k). Markedly, Ppara I-KO prevented the increase of villi length induced by cold treatment (Fig. 2l, Supplementary Fig. 6l), implying that PPARα is necessary for adaptive elongation of the crypt-villus axis, but dispensable for the longitudinal expansion of the small intestine.
upregulated in response to cold in sorted stem, Paneth, and transient progenitor cells in the crypts, as well as in sorted epithelial cells along the villus (Fig. 2n). Fractionation of intestinal tissue into villus, crypt and muscular-serosal layers confirmed that Ppara upregulation is limited to absorptive part of the intestine (Supplementary Fig. 7d). To check if HFD induces Ppara expression in the epithelial cells, in parallel to cold exposure, we isolated cells from Lgr5 mice fed HFD on RT, and confirmed predominant upregulation of Ppara isoform during HFD in jejunum, particularly in the progenitor cells in the crypts (Fig. 2n, Supplementary Fig. 7d).

**Intestinal Ppara KO reduces fat absorption and accumulation during HFD.** To investigate the functional role of intestinal PPARα in caloric absorption, we fed C57BL/6 J HFD (60% of calories from fat) or HF-HS diet (60% of calories from fat, 25% from sucrose) for six weeks. Both diets robustly increased calories from fat) or HF-HS diet (60% of calories from fat, 25% from sucrose) for six weeks. Both diets robustly increased

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**Fig. 2**

**a** Transcriptomics: Upregulated pathways in cold exposed mice in jejunum

**b** RNASeq rel. gene expression (RT chow vs. HFD)

**c** Metabolomic pathways in cold exposed mice in jejunum

**d** Coenzyme A metabolic pathway

**e** PPAR isoforms and regulators

**f** OGTT - Blood glucose (mM)

**g** ipGTT - Blood glucose (mM)

**h** ITT - Normalize blood glucose

**i** Fat pads weight (g)

**j** Small intestine length (cm)

**k** Jejunum - Perimeter (mm)

**l** Jejunum - Villi length (μm)

**m** Rel. gene expr. (Paneth)

**n** Lgr5-GFP+ (stem) CD24+ (Paneth) Lgr5-GFP+ (Paneth) Epcam+ (villus epithelium)
Energy harvesting pathways are upregulated in enlarged intestines of overeating mice, among which Ppara is necessary for villi elongation.

RNASeq pathway analysis by MetaCore upregulated pathways in jejuna of 30-day cold-exposed WT mice (Cold) compared to room temperature (RT) mice, n = 3 sample per group, each sample is a pool of two biological replicates. Relative RNASeq expression of metabolic genes in RT and Cold mice as in a, and in male ob/ob mice, n = 3 per group, where each sample is a pool of two proximal jejuna. RT n = 3 vs. Cold n = 3 (*), and WT n = 3 vs. ob/ob n = 3 (#) comparisons are from separate experiments, Counts normalized and RT and WT pooled together in the figure. Enriched metabolic pathways in Cold mice jejuna (the whole tissue lyse), for metabolite fold-change P < 0.05, as implemented in MetaCore, n = 5 mice per group. Relative concentration of eicosapentaenoic acid in jejuna of RT and Cold mice, n = 5 per group (P = 0.00594, z-scores of the replicates with a z-test, uncorrected for multiple comparison). RNASeq expression of PPAR-related genes in RT, Cold and ob/ob mice as in (b), together with the average count per million in the RT mice, " # " as in (b). f-m oral glucose tolerance test (f), intraperitoneal Gtt (g), insulin tolerance test (h), white adipose tissue pads (imgSAT inguinal subcutaneous adipose tissue, pgVAT perigonadal visceral) (i), small intestinal length (j), average perimeter of jejuna (k) and average villi length in jejuna (l) of 30-days cold-exposed Ppara lacx/lacx and Ppara-IKO male mice. 16 week-old, n = 6 mice per group. Gene expression by qPCR of Ppar isoforms in jejuna tissue of mice from (f-m). Gene expression by qPCR of adipocytes, or due to altered cell number along the villi, and villi length may be due to changes in the growth of individual enterocytes, or due to altered cell number along the villi, and found that PPARα deficiency reduced the cell number without affecting their size (Supplementary Fig. 8k, l).

Intestinal surface size depends on stem cell division and subsequent amplification of the progenitors in the intestinal crypts, and the effect of PPARα on the intestinal cell division suggests that this is necessary for intestinal crypt proliferation. Therefore, we addressed the role of PPARα in intestinal proliferation using organoid cultures. We isolated crypts from jejunum, and seeded them in matrigel and conditioned medium19. To investigate if the amount of budding new crypts from developed organoids is proportional to the in vivo intestinal surface size, we seeded crypts from +/ob and ob/ob mice. Organoids developed from ob/ob mice produced more crypts than the controls (Fig. 4a, b). This suggested that crypt-derived organoids in the culture retain proliferative properties of the source intestines. Next, we produced organoids from Ppara-IKO and control animals and found reduced budding in the KO organoids (Fig. 4c). We observed similar results by using a selective pharmacological PPARα antagonist GW-6471, where organoids derived from WT mice developed fewer crypts compared to the vehicle-incubated ones (Fig. 4d). To exclude potential PPARα-independent effects of GW-6471, we repeated the experiment in the Ppara-IKO mice and found no differences, suggesting that decreased budding is not due to cytotoxicity or non-specific targets (Fig. 4e). Another PPARα antagonist, NXC-629, produced similar inhibition of crypt budding in WT mice (Fig. 4f-h).

To test if the crypt proliferation depends on PPARα-driven fatty acid oxidation, we incubated organoids with etomoxir, an antagonist, NXT-629, produced more crypts than the controls (Fig. 4a, b). This suggested that crypt-derived organoids in the culture retain proliferative properties of the source intestines. Next, we produced organoids from Ppara-IKO and control animals and found reduced budding in the KO organoids (Fig. 4c). We observed similar results by using a selective pharmacological PPARα antagonist GW-6471, where organoids derived from WT mice developed fewer crypts compared to the vehicle-incubated ones (Fig. 4d). To exclude potential PPARα-independent effects of GW-6471, we repeated the experiment in the Ppara-IKO mice and found no differences, suggesting that decreased budding is not due to cytotoxicity or non-specific targets (Fig. 4e). Another PPARα antagonist, NXC-629, produced similar inhibition of crypt budding in WT mice (Fig. 4f-h).
Fig. 3 *Ppara* intestinal KO reduces adiposity, caloric uptake and postprandial lipidaemia on HFD. 

**a** Relative gene expression (qPCR normalized to *Tbp*) in proximal jejunum of WT C57BL6/J mice on ad libitum chow, HFD and HF-HS (*n* = 6 mice per group), *Ppara* lox/lox (*n* = 17), *Ppara* I-KO (*n* = 15) on HFD. 

**b** Daily food intake per mouse, *n* = 5 cages per group, two mice per cage on HFD. 

**c** Caloric density of faecal samples (d), daily fecal output (e), and total caloric uptake (calories from food – excreted calories) (f) in HFD mice, *n* = 5 (lox/lox) or four (I-KO) independent samples, where each sample is a pool of 48 h faeces from two mice in a single cage. 

**g–h** Fat pad weights, after four months of HFD (g), *n* = 14 mice per group from two independent experiments, and after 12 months of HF-HS diet (h), *n* = 7 (lox/lox) or 9 (I-KO) mice, ingSAT inguinal subcutaneous, pgVAT perigonadal visceral adipose tissue. 

**i** Triglyceride content in liver, *n* = 6 (lox/lox) or eight (I-KO) mice, and representative Oil red O staining of the liver cryosection, scale bar = 100 μm. 

**j** Relative gene expression (qPCR normalized to *Tbp*) in the liver of *n* = 4–9 (lox/lox) or 6–9 (I-KO) mice pooled from two independent experiments. 

**k** LDL, HDL and total cholesterol in plasma of fasted mice, measured by Cobas C111 platform, *n* = 5 mice per group. 

**l** Triglycerides (*n* = 12 mice per group) and free fatty acids (*n* = 11 mice per group) in plasma of fasted mice. 

**m, n** Plasma triglyceride levels after oral administration of 100 μl of olive oil (m) and corresponding area under the curve (n). *Ppara* lox/lox *n* = 10, I-KO *n* = 13. 

**o** Representative H&E staining and villi tracing, scale bar = 200 μm, and average jejunum villus length from *n* = 12 mice per group (o). 

*Ppara* lox/lox and *Ppara* I-KO are male mice on HFD (diet start at age eight weeks), sacrificed at age 25 weeks, if not indicates otherwise. 

**b, c** are representative of three independently repeated experiments, 

**e–o** are pool from two experiments. All data represent mean ± S.D., *P* ≤ 0.05, **P** < 0.01, ***P*** < 0.001 of unpaired two-sided t-test, confidence level 95%. Source data are provided as a Source Data file.
Fig. 4 Organoid crypt budding is decreased by PPARα knock-out and inhibition. 

**a** Number of crypts per organoid in the organoid cultures derived from two ob/− and two ob/ob mice on chow diet. 120 ob/− and 88 ob/ob organoids were analysed from 3 wells per group, at day 5, one-tail Mann–Whitney test. 

**b** Histogram of (a) n = 3 wells per group. 

**c** Histogram of organoid distribution according to the number of crypts from Ppara lox/lox and I-KO mice on chow diet, cultured without inhibitors, pool from two independent experiments and 6 (lox/lox) or 5 (I-KO) wells per group, total of 282 (lox/lox) or 220 (I-KO) organoids. One-tail Mann–Whitney tests on the histograms refer to the comparison of all organoids between the two groups (as in **a**), and asterisks for difference (two-tailed t-test) between frequencies of distribution on the histograms. 

**d** Histogram of organoid distribution by the number of crypts in the cultures from Ppara lox/lox, n = 3 wells per group (**d**) or Ppara I-KO mice (n = 5 wells for DMSO, n = 3 for GW-6471) (**e**), treated daily with DMSO or 5 μM GW-6471, on day 7. **f** Number of crypts per organoid, on day 9 of daily incubation with 2 μM NXT-629 and its vehicle, from two mice per group, 72 (vehicle) and 69 (NXT-629) organoids analysed, one-tail Mann–Whitney test. 

**g** Histogram of (**f**). 

**h** Representative images of organoid cultures from **d** and **f** on day 9, red astersisks mark crypt outgrowth, scale bar is 200 μm. 

**i** Violin plot of distribution of organoids per number of crypts in Ppara lox/lox and Ppara I-KO organoids when incubated with etomoxir, pooled from three wells per group on day 5 in a single experiment, median marked with solid, quartiles with dashed lines. Number of organoid, lox/lox: 251 (vehicle), 84 (2 μM), 169 (10 μM), 162 (50 μM), I-KO: 193 (veh.), 87 (2 μM), 270 (10 μM), 202 (50 μM). Number of crypts per organoid from ob/ob mice as in (a), on day 5 of daily incubation with a vehicle or 2 μM NXT-629, n = 88 (veh.), 127 (NXT), n = 201 (etomoxir). 

**j** Histogram of (**i**). 

**k** BrdU staining of jejunum 24 h after injection, with villi counterstained with Evan’s blue, and thin blue lines indicated measurements of villi and BrdU progression, scale bar is 200μm (**I**). BrdU progression along the villi in μm, and as a percentage of villus length (**o**), n = 6 (lox/lox), 7 (I-KO) mice per group. 

**l** Addition of WNT3A to basal ENR medium (EGF, Noggin, R-spondin) induces cystic crypts and organoids (red asterisks), potentiated by addition of palmitate, scale bar is 500μm. 

**o** Percentage of spherical organoids in the well, n = 3 per group, day 7. All data represent mean ± S.D. (except for **l**, as described) *P* < 0.05, **P** < 0.01, ***P** < 0.001 of unpaired two-sided t-test, confidence level 95%, except for (**a, f, and i**), where it stands for one-tail Mann–Whitney non-parametric test. Source data are provided as a Source Data file.
antagonism also reduced crypt budding in conditions of increased Ppara expression, both in the cultures from HFD (Supplementary Fig. 9a–d) and from ob/ob mice (Fig. 4j, k), whereas the proliferation in ob/ob organoids was reduced by etomoxir (Fig. 4j, k). In contrast, PPARα agonism by Wy-14643 increased proliferation on HFD (Supplementary Fig. 9e). Together, these data show that genetic and pharmacologic inhibition of PPARα reduces crypt proliferation capacity and indicate that fatty acid oxidation metabolically supports this process. To test if this is reflected in the proliferation in vivo, we injected mice with 5-bromodeoxyuracil (BrdU). Following 24 h, we detected decreased cell division and turn-over in the Ppara I-KOs compared to their control littermates (Fig. 4l, m). The reduced villi, but not small intestinal length in the Ppara I-KO mice indicate that PPARα is required for optimal adaptive growth of the crypt-villus axis. We found no difference in the frequency of Paneth, goblet cells or endocrine cells, nor in the most important gut hormones (Supplementary Fig. 10). Transcriptional data from enlarged guts and Ppara I-KO show that the markers of intestinal specification cell types and are mostly not affected at the transcriptional level (Supplementary Fig. 11).

A niche for stem cell proliferation is established by WNT proteins, excreted chiefly by Paneth cells, which trigger β-catenin signalling. Overnutrition, but also HFD and PPARα activation increase proliferation in the crypts via Wnt/β-catenin pathway. In our experiments, HFD increased β-catenin levels in whole jejuna, and also in proliferative progenitors (Supplementary Fig. 9f). To examine if PPARα may crosstalk with this pathway, we incubated organoids with exogenous WNT3A. Adding WNT3A favours the formation of round cystic organoids rich in stem cells and organoids with exogenous WNT3A. Adding WNT3A favours the formation in the intestines, we orally administered 100 μl of olive oil to mice that were fasted overnight. After one hour, mice were sacrificed and the sections of jejuna were treated with neutral lipid stains LipidTOX or Oil Red O (Fig. 6a, b). Quantification of lipid droplets in the intestinal epithelium revealed that the enterocytes of Ppara I-KO mice had diminished cytoplasmic lipid content, compared to epithelium of the control littermates (Fig. 6c). The lipid droplets of the Ppara I-KO mice were smaller than those of the controls (Fig. 6d), and these smaller droplets contributed more to total lipid droplet area than large droplets; in contrast to the controls (Fig. 6e). These observations are consistent with the attenuated postprandial lipaemia in Ppara I-KO mice at 1 to 2 hours after acute fat load (as in Fig. 3m). In Ppara I-KO jejuna, Cd36, Slc27a2 and Slc27a4 expression was decreased, whereas genes critical for glucose and peptide absorption, the re-esterification of absorbed fatty acids, packaging in chylomicrons, and mitochondrial content were not affected (Fig. 6f, Supplementary Fig. 9k, Supplementary Fig. 10m). In addition to increasing the expression of Acox1 and Pdka3, PPARα activation in tissue cultures also enhanced Plin2 (Fig. 5p), a lipid droplet surface protein in the intestines. Etoricox also increased Plin2, as noticed earlier45. On the other hand, peptide and fatty acid transporters Slc15a1 and Slc27a4 showed no (or only marginal) increase in expression. Nonetheless, inhibition of PPARα reversed these effects (Fig. 5q).

To investigate if there is a change in lipid uptake and droplet formation in the intestines, we orally administered 100 μl of olive oil to mice that were fasted overnight. After one hour, mice were sacrificed and the sections of jejuna were treated with neutral lipid stains LipidTOX or Oil Red O (Fig. 6a, b). Quantification of lipid droplets in the intestinal epithelium revealed that the enterocytes of Ppara I-KO mice had diminished cytoplasmic lipid content, compared to epithelium of the control littermates (Fig. 6c). The lipid droplets of the Ppara I-KO mice were smaller than those of the controls (Fig. 6d), and these smaller droplets contributed more to total lipid droplet area than large droplets; in contrast to the controls (Fig. 6e). These observations are consistent with the attenuated postprandial lipaemia in Ppara I-KO mice at 1 to 2 hours after acute fat load (as in Fig. 3m). In Ppara I-KO jejuna, Cd36, Slc27a2 and Slc27a4 expression was decreased, whereas genes critical for glucose and peptide absorption, the re-esterification of absorbed fatty acids, packaging in chylomicrons, and mitochondrial content were not affected (Fig. 6f, Supplementary Fig. 9k, Supplementary Fig. 10m). In contrast, Plin2 expression was strongly suppressed in Ppara I-KO, both at mRNA and protein level (Fig. 6f-h). PLIN2 (also known as adipophilin, ADRP) is critical for LD formation and stability, and its absence hampers the ability to form lipid droplets in many tissues46–48. Therefore, we further investigated the PPARα-dependant response of PLIN2 to palmitate load in intestinal organoids from Ppara I-KO and control mice. Palmitate increased PLIN2 protein levels over 8-fold in WT organoids. This increase was abolished in Ppara I-KO derived organoids.
Fig. 5 PPARα antagonism reduces fatty acid uptake and villi length in human intestinal biopsies. a Graphical representation of the human epithelial biopsy culture. b, c Basolateral (b) and apical radioactivity (c) of dipeptide [3H]GlySar, n = 7 per group of epithelial biopsy insets cultured seven days with PPARα antagonist 5 μM GW-6471 or DMSO. d, e Apical concentration of free fatty acids (d) and calculated palmitate absorption between 2 and 4 h, n = 7 (vehicle), 6 (GW-6471) biopsy insets (e). f Trans-epithelial electrical resistance at the end of the experiment, n = 7 (vehicle), six (GW-6471) biopsy insets. g, h Full villi lengths n = 88 (vehicle), 63 (GW-6471), pooled from n = 5 (vehicle), four (GW-6471) biopsy insets after nine days of treatment (g) and representative H&E staining with villi tracing (h). i, j Full villi lengths after nine days of incubation with PPARα antagonist 5 μM NXT-629 (n = 70 villi), or vehicle (n = 38 villi), pooled from four cell culture insets per group (i) and representative H&E staining of villi (j). k Palmitate absorption in 4 h, from n = 6 (vehicle) and four (NXT-629) insets per group, calculated from apical FFA concentration measurements. I Graphical representation of the mechanism of pharmacological PPARα agonism (Wy-14643) and antagonism (GW-6471), and of the fatty acid oxidation inhibitor etomoxir. m Fatty acid oxidation in human epithelial samples measured by scintillation counting of [14C]CO2 that is released by in vitro β-oxidation of [14C]-palmitate, n = 2 (DMSO, etomoxir), three (Wy-14643, Wy-14643-etomoxir). n-p Basolateral radioactivity of dipeptide [3H]GlySar after 4 h of transport (n), palmitate absorption in 4 h, calculated from apical FFA measurements (o) and relative qPCR gene expression in epithelial biopsies, normalized to Tbp (p) after 10-day inhibition with DMSO, 4 μM Wy-14643, 50 μM etomoxir, and Wy-14643 + etomoxir, n = 6 biopsy insets per group. q Relative qPCR gene expression after GW-6471 treatment, n = 7 (vehicle), eight (GW-6471) insets per group. b-h and o are pools from two independent experiments. All data represent mean ± S.D., *P ≤ 0.05, **P > 0.01, ***P < 0.001 of t-test confidence level 95%. Source data are provided as a Source Data file.

(with less than 1% remaining, Fig. 6h), demonstrating a near-complete depletion of PLIN2 upon PPARα deletion. Conversely, palmitate enhanced the Plin2 transcript for over 4-fold in WT organoids, while PPARα agonism led to over 20-fold increase of the Plin2 levels. Both palmitate and PPARα agonism had no effect on Plin2 expression in Ppara I-KO organoids (Fig. 6i), showing that the increase of PLIN2 after pharmacological activation of PPARα, and importantly upon palmitate stimulation, is PPARα dependant. Collectively, these findings suggest that lack of PPARα delays fat absorption through the intestine and that, at least partially, this is mediated by downregulation of perilipin 2, necessary for enterocyte lipid droplet formation.

Discussion

Our study addresses a fundamental question on the metabolic triggers and mechanisms regulating the plasticity and function of the intestinal lining. The work shows that the intestinal functional...
and morphological alterations depend on the food amount. Using multiple genetic models, and systematic in vivo approaches, we demonstrate that while several energetic pathways are dispensable, the intestinal PPARα-mediated lipid metabolism is of key importance for the adaptive villi lengthening and increased absorptive function in mice and human intestinal biopsies. This work proposes a concept in which the intestinal plasticity allows optimisation of caloric uptake from the consumed food, by coupling energetically costly intestinal epithelial maintenance to food availability.

Gut reshaping involves elongation, villus growth, microvilli extension, and upregulation of uptake transporters, though not all of them need to be employed equally and at the same time. These processes require energy resources. While our data show that gut expansion mobilizes glycolysis, gluconeogenesis and glutamate utilization pathways; there is a robust capacity for compensation in case one of them is affected. However, we found that PPARα is indispensable for adaptive villi growth and increased lipid transport. While fatty acid oxidation that is orchestrated by PPARα was needed for crypt-villus growth, its inhibition was insufficient to prevent intestinal lipid transport. Instead, our data suggest an alternative pathway, where the intestinal lipid droplet formation is reduced following PPARα depletion, coupled to a marked reduction of PLIN2, a key mediator of lipid droplet formation and growth. In DIO, PLIN2 is upregulated, forming ring-like structures around cytosolic lipid droplets as they grow, and Plin2 deletion affects lipid absorption in intestines and ameliorates DIO. In our experiments, PPARα deletion induced complete blunting of the Plin2 expression, and fully prevented its elevation driven by an increase in the fatty acid palmitate, which seemed sufficient to suppress the total TG absorption in line with data from the global Plin2-KO mice. This notion can be further supported by the current understanding of cytosolic lipid droplets representing a transitional pool of lipids that optimizes absorption during food consumption. Therefore, the reduced lipid uptake following PPARα inhibition may be a result of three complementary effects: decreased intestinal surface due to shorter villi, downregulation of fatty acid transporters on the apical surface of brush border, and reduced expression of Plin2 in the enterocytes. All three layers of regulation were considerably lower with inhibition or knock-out of Ppara, explaining the potent reduction of lipid uptake. These mechanisms also affect sugar and peptide transport, though ex vivo uptake assays and the respective transporter expression data suggest that their uptake is attenuated to a lesser extent than fatty acids.

We found no effect of PPARα deletion on the intestinal lipoprotein assembly machinery, but rather a decrease of fatty acid
uptake, packaging and export, independent of fatty acid oxidation status. Earlier reports suggested that docosahexaenoic acid31 and benzafibrate32,33, activators of PPARα and fatty acid oxidation, attenuate postprandial hyperlipidaemia in vivo and lower basal TG secretion in Caco-2 enterocyte cultures. It is important to note that these studies used exclusively pharmacological approaches, did not measure TG build-up and export in enterocytes after fat load, nor investigated for morphological changes on enterocytes, or contributions from other organs where the pharmacological PPARα activation would play a role. In line with our findings, it is known that ISC s are sensitive to dietary clues, and fatty acids provided by HFD increase ISC proliferation. This was recently attributed to activation of PPARα34, an abundant PPAR isoform in the crypt region, and to PRDM16, a transcriptional regulator of FACG34. However, deletion of PRDM16 did not alter villi or gut length16. Moreover, we found no or very small increase in its expression in any of the enlarged intestines, compared to the PPARα. As PPARα is more dominant isoform in duodenum, and PPARγ in jejunum (Supplementary Fig. 6e, g), it is possible that the two isoforms regulate self-renewal of different parts of intestine, and both PPARα and PPARγ depletion attenuate response to WNT3/β-catenin signalling, which reduces stem cell division and cell cycling. However, in contrast to Ppara, the Ppargc1a I-KO triggers increased fat gain during HFD35, indicating that the mechanism of fat handling is distinct in the two knockouts. The contribution of PPARα in the intestinal response to HFD was not addressed in these studies, but Hmargc2 is a well-known target of PPARα33,41, while PRDM16 is the upstream regulator of PPARα and other genes of FAO, especially the upper intestine. Fat-rich ketogenic diet activates HMGCS2 (the rate-limiting enzyme of ketogenesis) in ISC, which stimulates Notch signalling to promote ISC self-renewal56. In line with this, our data suggest PPARα as an indispensable mediator of intestinal homeostasis and cell division in jejunum, likely downstream of PRDM16.

It is important to note that various genetic and dietary models can have pleiotropic effects, which may contribute to the correlations observed between food intake and gut surface area. While our study points to the critical contribution of the food amount, it does not exclude that certain food components29 can affect the intestinal surface and function independently, or in concert with the food amount. Moreover, the transcription programs orchestrated by PPARα are indispensable for the villi growth in presence of dietary lipids, however, the direct link between the lipid metabolism and the food amount pends further investigations. As recently reported, PPAR and RXR signalling is involved in the complex transitions between early regeneration and latter differentiation in intestinal organoids57, and knock-down of RXR, a PPAR co-factor, reduced enterocyte generation, but increased the regenerative capacity. Intriguingly, while PPARα coordinates lipid metabolism and is upregulated by lipid-rich diets, there is no direct correlation between amount of ingested lipid and intestinal surface. Indeed, palmitate and HFD increase crypt formation and stem cell division without resulting in expansion of the absorptive surface (our data and16). All this shows that PPARα is essential, but not sufficient for gut surface increase, and that additional mechanisms are necessary to release the break on the intestinal surface expansion. In the case of overeating, this might involve activation of other energy pathways in concert with PPARα, and sensing of mechanical pressure in the intestines.

Mucosa thickening is a hallmark of intestinal adaptations that follow intestinal resection. It is, at the molecular level, a poorly understood process that includes bowel lengthening, growth of villi, and induction of transporter expression58. The PPARα-driven mechanism of villus growth could explain why fatty acids such as palmitate or linoleate are the most effective dietary therapy that promotes mucosa re-growth after bowel resection59,60, a process that can be further potentiated by feeding prostaglandins61. Fatty acids and eicosanoids are strong inducers of PPARα. Conversely, disruption of PPARα and fatty acid oxidation is found in celiac disease, a condition characterized by shortened villi and malabsorption62.

Hepatic steatosis and increased adiposity are major risk factors for the development of metabolic syndrome. PPARα ablation in the liver and adipose tissue facilitates fat accumulation in these tissues by reducing levels of fatty acid oxidation63. Unexpectedly, Ppara deletion in the intestine lowers fat uptake in the liver and fat. Intestinal lipid droplet formation and trafficking regulated by PPARα, therefore, represent important rate-limiting steps in systemic lipid metabolism. Inhibition of this process moderates the excursions of plasma triglycerides after a lipid-rich meal. If pharmacological inhibition of PPARα or lipid droplet formation could be limited to intestines, this would serve as an option for reducing fat absorption and hyperlipidaemia, but also total caloric uptake. As a whole, the remarkable gut plasticity that permits gut and villi to be shrunken or enlarged is, therefore, an asset as a reversible alternative to gastric bypass surgery, or other interventions that aim at reducing body weight gain and obesity-related complications.

Methods

Animals. C57BL/6J, ob/+ and ob/ob animals were purchased from Charles River France. Hk2 lox/lox mice (B6.129P2(Cg)-Hk2<tm1.1Uku) were purchased from Jackson Laboratory, crossed with Ppara lox/lox and, maintained on a specific pathogen free (SPF) environment.

C57BL/6J (Charles River, 60% kJ from fat, 6.0% of weight crude protein, 2.4 MJ/kg of metabolizable energy (ME)) was from ssniFF (Germany, E15742-34 (“blue pellets”). High-fat high-sucrose (HF-HS) diet, also known as Surwit diet (59% kJ from fats, 26% kJ from carbohydrates, 0.1% of weight crude fibre, 22.8 MJ/kg of ME), was from ssniFF, EF D12331 mod. (orange pellets). High-fat (HF) diet (60% kJ from fat, 6.0% of weight crude fibre, 21.4 MJ/kg of metabolizable energy (ME)) was from ssniff (Germany), E15742-34 (“blue pellets”). High-fat high-sucrose (HF-HS) diet, also known as Surwit diet (59% kJ from fats, 26% kJ from carbohydrates, 0.1% of weight crude fibre, 22.8 MJ/kg of ME), was from ssniFF, EF D12331 mod. (“orange pellets”). Energy-reduced diet was from ssniff (SM – Energy reduced, V9631-5170), and had energy density reduced by 40% compared to chow, mainly by replacement of starch with fibre (38% kJ from carbohydrates, 51% kJ from protein, 11% kJ from fat, 17.3% of weight crude fibre, 7.7 MJ/kg of ME). HFD no fiber diet in Supplemental Figure 1d, h, i, and Fig. 2n is from SAFE diets (France), 260 HF, food product U8978 Version 19, (60% kJ from fat, 6.0% of weight crude fibre, 23.1 MJ/kg of ME). Delta fibre (control) and added fibre (HF-Delta fiber) are nearly identical diet, with added 1.4% cellulose (22.2 MJ/kg of ME). Additionally, chow diets from SAFE diets (SAFE-150) and chow (V1554-703) were used in

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Animal procedures and measurements. Male 14 to 18-week-old Ppara mice were placed in the calorimetric cages (Labnavigator, TSE, BadHomburg, Germany) to measure food intake, locomotor activity, oxygen consumption and production during seven days; energy expenditure was normalized to lean mass. For acute fast load test, mice were fasted for 3 h and gavaged with 100 μl of olive oil (Sigma-Aldrich). Blood samples during oral and intraperitoneal glucose tolerance tests (OGTT, IPGTT) and acute fast load test were collected from the tail vein in EDTA coated tubes provided in the Supplementary Table 1. For OGTT or IPGTT, mice were fasted 12 h overnight and gavaged or injected, respectively, with 2 ml of glucose per g of body weight. For bomb calorimetry of the faeces, 48-hour faeces were collected from the cage bedding (2 mice per cage), vacuum dried, pulverized to the fine powder and weighted in the calorimeter (Parr, 6100, USA). Consumed energy was calculated by formula: digestible energy (kcal) = food intake (g) x food calorific density (kcal/g) – faeces weight (g) x faeces calorific density (kcal/g).

Tissue and blood processing and analysis. Before sacrifice, mice were fasted 5 hours (unless specified otherwise). Blood collection. 500 μl of blood was taken from terminally anesthetized mice in tubes with 11 μl of 0.5 mM EDTA, 4 μl of aprotinin (1.3%) and 4 μl of DFP-Iv (10 mM) and plasma stored at −80 °C. GLP-1, GIP, glucagon and ghrelin were measured by Bio-Plex Pro Mouse Diabetes panel (1#701601), from plasma diluted 1:4 according to instructions, on Bio-Plex 200 Luminex platform (Biorad). Triglycerides were measured by Trig/GB kit (Roche), free fatty acids by NEFA-HR kit (Wako). Intestines were carefully unfolded and samples taken ensuring that they are from the same sites between different mice. RNA isolation and qPCR. RNA from tissues and cell cultures was isolated with TRIzol® Reagent (Thermo Fisher) and cDNA synthesized by High-Capacity cDNA Reverse Transcription Kit (Applied Biosciences). Transcript levels were measured by qPCR as indicated in the legend, the counts were normalized according to the housekeeping gene 18S and † indicated.

Microscopy. Tissues for haematoxylin and eosin staining (H&E) and immunohistochemistry were saved in 4% paraformaldehyde (PFA), and for electron microscopy 2.5% glutaraldehyde in cedacolate buffer. For lipid droplet visualization and quantification, the liver and proximal to middle jejunal samples were frozen on dry ice, cryosectioned, fixed briefly in 4% PFA and treated with Oil Red O with haematoxylin counterstain, or with LipdTOX HCS LipdTOX® Green Neutral Lipid Stain (Life Technologies) and DAPI (blue) nuclear stain. Images were taken from Oil Red O staining, in Fiji Image software 1.51 by thresholding stained scars in red channel, then automatic counting of lipid droplets and measuring their area, then normalizing to total epithelial tissue area (measured by thresholding it for red channel, then automatic counting of lipid droplets and measuring their area) per millimeter length, and 10 villi per section from 2 mice were counted. CD31+ and CD24high/Sidescatterhigh EGFP+ cells were analyzed in the samples from the proximal jejunum by the transmission electron microscope Morgagni (FEI Company, Eindhoven, Netherlands) at EM Core Facility of University of Geneva. 40–80 electron micrographs per section, taken from the most mineralized part of multilayered tissues from P86 with PBS in 2 mm EDTA. Metabolomics samples were collected by pipetting up and down, counted and sealed 75 μl in 20 μl of Matrigel (Corning 356231 growth factor reduced) per well of flat-bottom 48-well plate. After solidification, 300 μl of ENR (EGF, epo, insulin) medium was added in which the organoids were cultured. Advanced DMEM/F12 medium, supplemented with EGF 40 ng/ml (Peprotool), recombinant murine Noggin 200 ng/ml (Peprotool), R-spondin (a gift from Gerald Schwank, University of Zurich; 5 ml of R-spondin conditioned medium from 293T-HEK-RspoFc cell line per 50 ml of the culture medium), 1 μM N-acyl-L-cysteine (Life technologies), 1X N2 (Life Technologies), 1X B27 supplement (Life Technologies). For organoids in medium, murine WNT3A (Peprotech) was added to ENR in the following concentrations: 50 ng/ml, 150 ng/ml and 500 ng/ml. When the concentration indiated. Treatment with drugs started on second day after seeding with 2 μM GW-6471 (Sigma-Aldrich), 2 μM NXT-629 (MedChemTronica), 4 μM Wy-14643 (Sigma-Aldrich), 50 μM etomoxir (Lucerna), 200 μM palmitate/BSA coated plates (Yamada, MatTek) were used. The concentrations are given in each experiment. 60 organoids were counted per well, and mean and standard deviation were calculated.

Flow cytometry (FACS). Cysts from Lgr5-GFP mice were isolated as above, and suspension of vili in PBS was obtained by gently scraping longitudinally opened section of proximal-to-mid jejunum by microscope cover glass. Cell suspension was spun down, resuspended in 1 ml of undiluted TrypLE Express (Invitrogen) + 100 μl of DNase I (10 U/μl, Roche), then incubated in a 32 °C thermostock for 2 min, without shaking, and placed on ice. Pelleted cells were washed once in Advanced DMEM/F12, and incubated 30 min on ice in antibody mix of 1 μl of each CD45-PE (eBioscience, 30-F11), CD31-PE (Biogene, Mec13.3), Ter119-PE (Biogene, Ter119), CD24-Pacific Blue (Biogene, M1/69), and EPCAM-APC (eBioscience, G8.8) in 1 ml of Advanced DMEM/F12. Cells were washed twice in PBS and filtered through 70 μm mesh. 7-aminoactinomycin D (7-AAD) (7-ADD) was added to cell suspension to label dead cells. For software BD FACS Diva Software version 8.0.2. CD31+ CD45− Ter119− 7-AAD+ were excluded, others isolated as follows: intestinal stem cells: Lgr5-GFPhighEpCAM− CD24lowNXT-629− transiently amplying progenitors: EGFhighEpCAM− CD24highNXT-629− Paneth cells: CD24highSidescatterhighEGFP− EpCAM−; epithelial cells from vili: epithelial marker (Vimentin, Chromogranin A (sc-1488, Santa Cruz), Alcian blue was used as previously detailed. CD31+ EpCAM− paneth cells were incubated with primary antibody mix (anti-CD31+ and anti-EpCAM) and then with Alexa Fluor 647 anti-mouse (Calbiochem). Cells were sorted in 1 μl FACS Tysm version 10.7. Cells were sorted in RTL solution (Qiagen). RNA was isolated from the pellets with RNeasy® Micro Kit (Qiagen) according to the manufacturer’s instruction for isolation from a cell suspension, and cDNA synthesized by High-Capacity cDNA Reverse Transcription Kit (Applied Biosciences).

Human epithelial cultures. Human intestinal 3D reconstituted epithelial biopsies were obtained from a commercial supplier MatTek Life Sciences Europe (Bohemia, Czechia) (MatTeK Life Sciences). Biopsies were sequentially cultured on human cells and cultured in accordance with applicable regulations and guidelines including General Data Protection Regulation (Regulation (EU)2016/679), are fully anonymized, and the study was conducted in accordance with Declaration of Helsinki on biomedical research. Biopsy cultures were maintained in proprietary medium supplanted on day 2 on apical side with the inhibitors in the concentrations described in the previous section, and renewed every day. For glucose uptake assay: 30 mM D-glucose spiked with 0.1 μCi/mM of D/[4-14C]-glucose (specific activity 55 mCi/mM, Amersham, Switzerland) was applied to apical side of a tissue inset. For didepideptide uptake assay: 20 μM of glycyl-sarcosine (Sigma-Aldrich), spiked with glycyl-[2-3H]-sarcosine, and apical and basolateral medium sampled at 0, 1, 2 and 4 hours. For palmitate absorption, 400 μM of palmitate was used to label tissue samples. Microvilli were imaged in the samples from the proximal jejuna by the transmission electron microscope.
L-carnitine hydrochloride, 200 μL of palmitic acid, spiked with 0.5 μCi/mL of palmitic acid (Anawa, [1-14C] / s.A, 50-60 mCi/mmol, Conc. 0.5 mCi/ml), at 37 °C. CO2 released was captured on the filter paper soaked in 1 M NaOH and placed in the cap tube. Filter paper was put in 3 ml of the scintillation liquid (PerkinElmer Ultima Gold) and radioactivity was measured in the scintillation counter, then normalized to the input protein. Before the harvest, the integrity of barrier function was confirmed by measuring trans-epithelial electrical resistance with a cell culture voltrohmeter (EVOM, World Precision Instruments). Membranes with the tissues were excised from the insets, cut in half and saved for RNA isolation and histology.

**Statistical analysis.** Statistical tests are specified in the figure legends. To calculate significances, we used: for normally distributed continuous data (e.g. body weight, plasma triglycerides, qPCR etc.) non-parametrical tailed Mann–Whitney rank test with confidence level of 95%; for the discrete data (crypts per organoid) non-parametric one-tailed Student t-test with confidence level of 95%; for the multiple comparisons, we used non-parametrical one-way ANOVA, with Dunnet post-hoc correction, the alpha 0.05. For the gene expression levels by RNA sequencing, significances are calculated by general linear model with negative binomial distribution. P values without correction are shown in the figures. For pathway enrichment analysis, transcripts with log(count per million reads) >1 and P < 0.05 were selected and run through MetaCore software pipeline ( Thomson Reuters, build 6.21.66768, portal.genego.com). Simple linear regression was used to calculate correlations and Pearson’s R2 coefficient, with confidence intervals 95% and two-tailed P value at alpha 0.05. Graphs and statistical analysis were done in GraphPad Prism 8.3.

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

**Data availability**

Sequence data associated with this study have been deposited to the Gene Expression Omnibus with accession codes GSE47428, GSE182348, and GSE150256. Source data are provided with this paper. Metabonomic source data are provided in the Supplementary Data 1 of the paper. All other data used in this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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Author contributions
O.S. designed and performed the experiments, analysed the data and wrote the manuscript. J.A. and F.R.J. produced and initially characterized Ppara I-KO mice. S.F. obtained the initial ob/ob data, and P.M. contributed db/db data and Glut1 lox/lox mice. D.R. helped with the experiments, prepared samples for histology and acquired electron micrographs. M.S., E.E. and B.R. helped with the experiments. N.Z. measured metabolites. M.T. initiated and guided the study, analysed and interpreted the data and wrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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