Peroxisome Proliferator-Activated Receptor γ2 Controls the Rate of Adipose Tissue Lipid Storage and Determines Metabolic Flexibility

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In Brief
Virtue et al. describe how the transcription factor peroxisome proliferator-activated receptor gamma 2 (PPARγ2) regulates lipid storage rate in adipose tissue. Mice lacking PPARγ2 cope when fat storage demands are low, but acute overfeeding overwhelms adipose tissue, redirecting lipid to muscle and causing insulin resistance.

Highlights
- Mice lacking PPARγ2 have impaired adipose tissue lipid storage rate
- Low adipose tissue storage rate leads to metabolic inflexibility
- Acute hypercaloric challenges can detect impaired adipose tissue lipid storage rate
- Chronic adipose tissue metabolic inflexibility leads to insulin resistance with age
Peroxisome Proliferator-Activated Receptor γ2 Controls the Rate of Adipose Tissue Lipid Storage and Determines Metabolic Flexibility

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SUMMARY

One understudied function of white adipose tissue (AT) is its role in postprandial lipid buffering. In this study, we demonstrate that mice lacking the adipose tissue-specific transcription factor peroxisome proliferator-activated receptor γ2 (PPARγ2) exhibit a defect in their rate of adipose tissue lipid storage. Impaired adipose tissue storage rate reduces metabolic flexibility, without compromising fasting glucose tolerance or insulin sensitivity, even following prolonged high-fat feeding. However, acutely overfeeding PPARγ2-KO mice caused a 10-fold increase in insulin levels compared with controls. Although impaired adipose tissue storage rate did not result in insulin resistance in young mice, 1-year-old PPARγ2-KO mice developed skeletal muscle insulin resistance. Our data indicate that failed adipose tissue storage may occur prior to defects in glucose handling and that overfeeding protocols may uncover genes controlling adipose tissue storage rate, as opposed to capacity, and act as a diagnostic test for early-stage human metabolic disease.

INTRODUCTION

The epidemic of obesity has led to epidemics of diabetes and cardiovascular disease. An important question is how obesity leads to metabolic complications. A proposed mechanism linking obesity to metabolic complications is the concept of adipose tissue (AT) expansion and lipotoxicity. The adipose tissue expansion hypothesis suggests that metabolic complications occur when an individual’s adipose tissue storage capacity is exceeded (Virtue and Vidal-Puig, 2010), that preventing adipose tissue expansion will worsen metabolic complications (Danforth, 2000), whereas unlimited adipose tissue expansion would be metabolically protective, a concept demonstrated in mice (Kim et al., 2007).

We previously reported (Medina-Gomez et al., 2005) the phenotype of mice lacking peroxisome proliferator-activated receptor γ2 (PPARγ2). PPARγ2 is the adipose tissue-specific isoform of PPARγ. Surprisingly, despite defective adipogenesis in vitro, mice lacking PPARγ2 had normal adipocyte size on a Chow diet (Medina-Gomez et al., 2005), as well as normal glucose and insulin tolerance on Chow or chronic high-fat (3 months of feeding) diets. However, crossing the PPARγ2-knockout (KO) mouse onto an Ob/ob background (POKO mouse) led to severe metabolic syndrome. Importantly, defects in insulin sensitivity in POKO mice occurred before any divergence in adiposity between genotypes (Medina-Gomez et al., 2007). The early onset of metabolic impairments in POKO mice raised the question as to which aspect of leptin deficiency was driving the phenotypic divergence. Furthermore, why PPARγ2-KO mice on a non-obese background were metabolically healthy was unresolved.

Although PPARγ2 KO mice had normal-sized adipocytes on a Chow diet, they showed molecular markers of impaired adipocyte function (Medina-Gomez et al., 2005). Dysfunctional adipose tissue function has been suggested to lead to impaired metabolic flexibility in both humans (Frayn, 2002) and rodents (Asterholm et al., 2012; Asterholm and Scherer, 2010). Metabolic inflexibility is the inability to switch efficiently between predominantly using lipid in the fasted state to carbohydrate in the fed state (Goodpaster and Sparks, 2017; Storlien et al., 2004). In humans, obesity is characterized by low metabolic flexibility (Storlien et al., 2004). Although direct defects in muscle lipid and glucose use have been suggested to cause metabolic inflexibility (Ukropcova et al., 2007), defects in adipose tissue function can also cause metabolic inflexibility by altering the fluxes of lipids to muscle (Frayn, 2002). Dysfunctional adipose tissue fails to store postprandial lipid and redirects it to non-adipose organs. Conversely, in the fasting state, insufficient release of free fatty acids (FFAs) by adipose tissue occurs because of impaired lipolysis (McQuaid et al., 2011).

Although multiple studies have investigated metabolic flexibility in mice (Asterholm et al., 2012; Asterholm and Scherer, 2010; Gao et al., 2015; Isken et al., 2010; Jackowski and Leonardi, 2014; Longo et al., 2008; Muoio et al., 2012; Riachi
multiple small meals per day (Ellacott et al., 2010), and as a
result, the rate at which the adipose tissue of mice is required
to store and release lipid maybe relatively low. In contrast, hu-
mans tend to eat three large meals a day, causing fewer more
acute demands for adipose tissue lipid storage (McQuaid
et al., 2011). Overall, a loss of adipose tissue flexibility may man-
ifest differently in rodents and humans.

The most commonly used model for inducing obesity and
insulin resistance in mice is high-fat diet (HFD) feeding. HFD
causes many mouse strains to consume more calories and
gain weight, but after an initial period of rapid body weight

gain, usually lasting less than 1 week, body weight divergence between HFD
and chow-fed mice tends to be relatively slow. The slow rate of weight gain caused
by established HFD suggests that chronic HFD may not actually greatly in-
crease demands on adipose tissue stor-
age rate.

In this study, we reinvestigated
PPARγ2-KO mice. Although PPARγ
is traditionally viewed as a regulator of adi-
pogenesis, PPARγ2 is regulated by fast-
ning and refeeding (Vidal-Puig et al.,
1997), suggesting an additional dynamic
role for PPARγ2. Supportive of this
concept, PPARγ2 is necessary for appro-
priate expression of lipolytic genes in ad-
ipoocytes (Rodriguez-Cuenca et al., 2012). In this study, we
demonstrate that PPARγ2-KO mice have a reduced adipose tis-
sue lipid storage rate and exhibit metabolic inflexibility. In states
in which adipose tissue lipid storage rates are low, mice lacking
PPARγ2 have normal insulin and glucose levels. To challenge
adipose tissue storage rate, we exploited the fact that mice
failed to store lipid in adipose tissue and redirect it to mus-
cle, causing triglyceride (TG) accumulation and insulin resis-
tance, with PPARγ2-KO mice exhibiting 10 times the insulin
levels of controls. Importantly, mice lacking PPARγ2 developed
insulin resistance with aging, suggesting that a primary loss of
adipose tissue lipid buffering may be initially tolerated but
ultimately leads to metabolic disease. Finally, we demonstrate that, in line with a potential acute metabolic role for PPARγ2 in adipocytes, PPARγ2 but not PPARγ1 correlates with whole-body and adipose tissue insulin sensitivity in humans.

RESULTS AND DISCUSSION

PPARγ2-KO mice had normal fasting glucose tolerance (Figures 1A–1C) (Medina-Gomez et al., 2005), insulin levels (Figure S1A), glucose infusion rate (GIR), rate of whole-body glucose disappearance (RD), and hepatic glucose production (HGP) during a euglycemic-hyperinsulinemic clamp (Figure 1D). However, PPARγ2-KO mice had reduced metabolic flexibility in the fed state (Figures 1E–1G). On the basis of their amplitude of respiratory exchange ratio (RER), PPARγ2-KO mice had increased glucose oxidation in the light phase and increased lipid oxidation in the dark phase relative to controls (Figure 1F). To investigate the increased glucose oxidation in the light phase, we performed a glucose tolerance test (GTT) in the fed state at 10 a.m., incorporating stable isotopes. Consistent with their RER profile, mice lacking PPARγ2 exhibited increased clearance of exogenous glucose (Figures 1H–1J). The PPARγ2-KO mice also exhibited reduced RER during the night phase, suggesting that PPARγ2 mice may have impaired adipose tissue lipid storage leading to excess delivery of lipid to muscle (Randle et al., 1963), and in line with this concept, mice lacking PPARγ2 were unable to clear an oral lipid load as effectively as controls, exhibiting higher TG (Figure 1K) and FFAs (Figure S1B) during a lipid tolerance test.

In response to chronic HFD, mice lacking PPARγ2 have normal carbohydrate metabolism and adipose tissue mass (Medina-Gomez et al., 2005). However, given their compressed RER profiles and inability to clear lipids, we hypothesized that PPARγ2-KO mice may exhibit a defect in the rate of adipose tissue lipid storage, rather than total storage capacity. This hypothesis was also supported by the early onset (4 weeks of age) of hyperinsulinemia in POKO mice, an age at which ob/ob mice are already hyperphagic but prior to a divergence in adiposity relative to controls (Medina-Gomez et al., 2007). To stress adipose tissue storage rate, we developed an overfeeding challenge by giving mice a high-fat diet for 1 day (1dHFD). We first characterized 1dHFD in wild-type mice. Mice on 1dHFD doubled food intake (Figure S1C) and increased insulin levels (Figure S1D) to maintain normoglycemia (Figure S1E). Body weight and subcutaneous white adipose tissue (scWAT) mass were increased (Figures S1F–S1H). Finally, Pparγ2 expression in scWAT was physiologically increased in response to 1dHFD, suggestive of a role for PPARγ2 in responding to overfeeding (Figure S1I).

PPARγ2-KO mice placed on HFD for 1 day exhibited similar food intake (Figure 2A), diet-induced thermogenesis (Figure 2B), and body weight gain (Figure 2C) to controls, whereas absolute body weight, BAT weight, liver weight, and liver fat percentage were increased (Figures 2D–2F).

When subjected to 1dHFD, PPARγ2-KO mice exhibited dramatically increased insulin levels and increased glucose and FFA levels compared with 1dHFD wild-type controls. Importantly, prolonged HFD feeding (for either 1 week or 1 month) normalized the serum biochemistry parameters of PPARγ2 mice (Figures 2G–2J, S1L, and S1M), even though PPARγ2-KO mice after 1 month of HFD had greater adiposity (Figures S1J.

**Figure 2. PPARγ2-KO Mice Exhibited Impaired Metabolic Responses to Acute Overfeeding**

(A–F) Responses to 1 day high-fat diet (1dHFD): (A) food intake, (B) change in energy expenditure (dEE), and (C) change in body weight (dBW) between chow and after 1dHFD; (D) body weight, (E) tissue weights, and (F) liver fat percentage after 1dHFD period.

(G–J) Serum biochemistry for (G) insulin, (H) TGs, (I) FFAs, and (J) glucose after 1dHFD. N = 8 per group except 1 month HFD KO and food intake for chow and 1dHFD WT (n = 6). *p < 0.05, two-tailed Student’s t test. All mice were 4 months of age. All data are represented as mean ± SEM. See also Figures S1 and S2.
Figure 3. Mice Lacking PPARγ2 Cannot Appropriately Store Lipid in Adipose Tissue

(A–C) Gene expression in WAT for (A) lipoprotein lipase and regulatory molecules and (B) FA uptake genes. (C) Markers of lipolysis (n = 8 mice per group except 1 month HFD KO [n = 7]).

(D and E) Western blots of WAT: (D) representative western blots and (E) quantification (n = 8 mice per group).

(legend continued on next page)
and S1K) than PPARγ2-KO mice after 1dHFD (Figure 2E). Finally, we conducted a fasting-refeeding experiment and demonstrated that mice lacking PPARγ2 had greatly increased insulin in response to refeeding after an overnight fast (Figures S2A and S2B). Overall, our data suggested a lack of PPARγ2 impaired adipose tissue lipid storage rate and a failure in adipose tissue storage rate caused insulin resistance in response to overnutrition.

We next sought to confirm that PPARγ2-KO adipose tissue was unable to buffer lipid appropriately. We first analyzed gene expression in scWAT. In terms of genes controlling hydrolysis of TG to FFAs, lipoprotein lipase (Lpl) tended to be upregulated, whereas the LPL inhibitory protein angiopoietin-like 4 (Angptl4) was downregulated in PPARγ2-KO scWAT after 1dHFD (Figure 3A). The FFA transporter-encoding genes FA transport protein (Fatp1) and Cd36 were downregulated in PPARγ2-KO mice by 1dHFD (Figure 3B). The trend to upregulation of Lpl and the downregulation of Angptl4, Cd36, and Fatp1 were consistent with the normal TG levels and increased FFAs in PPARγ2-KO mice following 1dHFD. To exclude a role for lipolysis in regulating circulating FFA levels, we analyzed expression of adipose TG lipase (Atgl), which was downregulated under high-fat feeding conditions and hormone sensitive lipase (Hsl), which was downregulated under all nutritional conditions (Figure 3C) in PPARγ2-KO mice. HSL phosphorylation at the activating site 6660 was decreased in the scWAT of PPARγ2-KO mice following 1dHFD (Figures 3D and 3E), while insulin signaling, a known suppressor of lipolysis, was either similar (ratio of phospho-AKT to total AKT) or upregulated (ratio of phospho-P44/42 ERK to total P44/42 ERK), in line with the increased circulating insulin levels. Overall, and in line with our previous work (Rodriguez-Cuenca et al., 2012), the elevated FFAs in PPARγ2-KO mice were unlikely to be a result of FFA release by adipose tissue but a result of a defect in FFA uptake.

To confirm a defect in FFA transport into adipose tissue, we used the fact our HFD has an increased nonessential (NE)/essential (E) FA ratio compared with chow. Feeding mice an HFD leads to a progressive increase in NE/E FA ratio in white adipose tissue (WT) (Yew Tan et al., 2015). Wild-type (WT) and PPARγ2-KO mice had similar NE/E FA ratios under chow conditions, but 1 day HFD increased NE/E FA ratio only in WT mice (Figures 3F and 3G), a difference that was normalized between genotypes after 1 month of HFD. Importantly, this analysis detects existing fat as well as newly stored fat. The 15% increase in NE/E ratio in WT mice was in good agreement with the 20% increase in fat mass observed in response to 1dHFD (Figure S1G), suggesting that most of the fat accumulating in the scWAT of WT mice was dietary derived. Further support for a lack of lipid uptake to adipocytes was provided by histological analysis of WT and PPARγ2-KO mouse adipocyte sizes. Although WT mice increased their adipocyte size following 1dHFD, PPARγ2-KO mice did not (Figures 3G and 3H). Finally, we traced palmitate uptake to WAT under euglycemic-hyperinsulinemic clamp conditions. PPARγ2-KO mice exhibited increased 14C-palmitate in blood (Figure 3I) and decreased incorporation into adipose tissue (Figures 3J and S2D–S2F). Overall, these results suggested that elevated circulating FFA levels in PPARγ2-KO mice were caused by a failure to uptake and esterify FFAs into WAT, a concept further supported by the elevated FFAs during a lipid tolerance test (LTT) (Figure S1B).

The failure of adipose tissue to clear FFAs (Figure 3J) suggested a potential increased flux of FFAs to other organs (Virtue and Vidal-Puig, 2010), which we next sought to investigate. In liver, mice lacking PPARγ2 exhibited a more lipogenic response to acute overfeeding than WTs (Figures S2G–S2K), potentially reflecting the increased insulin levels (Figure 2G), but no differences in FA oxidation markers or changes in the phosphorylation status of insulin signaling molecules (Figures S2L and S2M). Similarly to liver, no defects in brown adipose tissue (BAT) insulin signaling (Figures S3A and S3B), or glucose disposal into BAT or WAT (Figure S3C) were detected. Conversely, AKT phosphorylation was impaired in skeletal muscle after 1dHFD in PPARγ2-KO mice (Figures 3K and 3L). Muscle gene expression showed that several oxidative metabolism genes in PPARγ2-KO mice (Figure 3M) reached levels of expression after 1dHFD that were similar to or greater than those observed for either WT or PPARγ2-KO mice after 1 month of high feeding. Additionally, lipid uptake and TG storage genes tended to be increased in the muscle of PPARγ2-KO mice compared with controls (Figure 3O). HFD did not modify mRNA markers of insulin sensitivity in PPARγ2-KO muscles (Figure 3N) or markers of lipogenesis (Figure 3P) between genotypes.

To validate gene expression changes, we analyzed muscle lipid composition. On a chow diet, WT and PPARγ2-KO mice had similarly large muscle lipidomes (Figure S3D) that could not be separated using multivariate analyses (Figures 4A and 4B), but following 1dHFD, a significant separation in the lipid profiles was detectable (Figures 4A and 4B). PPARγ2-KO mice accumulated more longer chain FA-containing TGs than controls (Figure 4C). Finally, we measured the acylcarnitine profile of the muscles. Under chow conditions, and consistent with their increased carbohydrate oxidation in the light phase, mice lacking PPARγ2 had less short-chain acyl carnitines, but this phenotype was reversed by 1dHFD (Figures 4D and S3E). In summary, our data supported the idea that PPARγ2-KO mice exhibit a...
primary defect in adipose tissue storage rate, which in the context of acute overfeeding drives FFA flux to muscle. Increased FFA flux to muscle leads to lipid accumulation, lipotoxicity-induced insulin resistance, and compensatory upregulation of the FA oxidation program (Randle et al., 1963).

Although 1dHFD was able to unmask the underlying metabolic defects of PPARγ2 ablation, even under chow conditions, mice lacking PPARγ2 had impaired metabolic flexibility (Figure 1E) and increased expression of the lipid oxidation genes Aox and muscle insulin resistance, with aged PPARγ2-KO mice exhibiting lower RD, reduced GIR, and normal HGP (Figures 4J–4L) as well as increased fed insulin levels (Figures S3F–S3J) compared with WTs. Adipose tissue showed relatively little further impairment with aging compared with young animals (Figures S4A–S4E), and liver exhibited almost no changes between PPARγ2-KO and WTs (Figures S4F–S4J).

The human relevance of our observations was confirmed in adipose tissue from 45 obese subjects who had undergone a

Figure 4. Loss of Adipose Tissue Lipid Buffering Leads to Aging-Related Insulin Resistance

(A) Principal-component analysis of lipid species from muscle of chow (left) or 1dHFD (right) fed mice. (B) Quantification of the first two principal components from (A). (C) Muscle TG species. (D) Muscle acylcarnitines species (n = 6–8 per group). (E–I) Gene expression in muscle of 1-year-old mice: (E) F4/80, (F) FA oxidative markers, (G) lipid uptake and storage genes, (H) lipid biosynthetic genes, and (I) Insulin sensitivity markers (n = 10 WT, n = 7 KO, male mice chow fed). (J–L) Clamps from 1-year-old mice: (J) rate of glucose disposal (RD), glucose infusion rate (GIR), and hepatic glucose production (HGP) under hyperinsulinemic condition; (K) suppression of HGP between the basal and hyperinsulinemic state; and (L) body weights of mice used for clamps (n = 7 WT and 6 KO mice, chow fed). (M–O) Correlations between PPARγ2 expression in human scWAT and (M) M-value during a clamp, (N) IRS1 expression, and (O) Glut4 expression (n = 45 subjects). For multiple time points, two-way ANOVA was performed, and if significant, pairwise comparisons were performed using two-tailed Student’s t test (*p < 0.05). Correlations are Pearson’s, and exact p values are reported. All mice were 4–5 months old. All data are represented as mean ± SEM. See also Figures S3 and S4.

Cpt1b (Figure 3M) in skeletal muscle. To determine if a loss of metabolic flexibility due to adipose tissue dysfunction led to longer term metabolic complications, we aged PPARγ2-KO mice and controls on a chow diet for 1 year. Aged mice lacking PPARγ2 exhibited decreased expression of Pgc1α (Figure 4F) and insulin sensitivity markers including the FA synthesis program and Irs1 (Figures 4H and 4I) and increased expression of the macrophage marker F4/80 (Figure 4E) in muscle. Notably, other than a tendency toward reduced Scd1 expression, these changes were absent in the muscles of young mice on a chow diet (Figures 3M–3P). These molecular changes manifested in skeletal
Our study identifies PPARγ2 as a crucial regulator of adipose tissue lipid storage rate, in addition to its role in adipose tissue development and total storage capacity. A reduction in adipose tissue storage rate limits the ability of adipose tissue to respond acutely to large nutrient influxes and causes metabolic inflexibility that chronically leads to insulin resistance. Our data therefore suggest a possible evolutionary rationale for the presence of PPARγ2 in mature adipocytes, as a nutritional integrator of lipid uptake and release that controls the availability of FFAs (Rodriguez-Cuenca et al., 2012) and gluconeogenic substrates (Perry et al., 2015; Titchenell et al., 2016) in the fasted state and permits appropriate lipid storage in the fed state. Because the mouse model of PPARγ2 ablation we have used is a total KO, we cannot exclude effects of PPARγ2 in other organs, but PPARγ2 is almost exclusively expressed in adipose tissue (Escher et al., 2001). Furthermore, although it is possible that effects on lipid uptake and release could be secondary to alterations in adipogenesis, we observed these defects in chow-fed mice at an age when PPARγ2-KO animals possess normal adipocyte numbers (Figures 3G and 3H).

Under laboratory conditions of ad libitum access to food, mice do not eat like humans, consuming many small meals throughout the day (Eliaconn et al., 2010). Conversely, most humans consume a small number of large meals a day and occasionally tend to over-eat. As such, the nutrient load that humans face is more infrequent, with a larger and more variable amplitude than that faced by mice. Although not perfectly analogous to the human breakfast-lunch-dinner feeding pattern, the 1dHFD model in rodents increases the amplitude of their nutrient intake and importantly keeps them in circadian phase. The 1dHFD model may therefore provide a more translatable metabolic challenge than traditional chronic HFDs for the study of adipose lipid storage function, and it is likely that other mouse models with similar defects maybe uncovered using a 1dHFD protocol.

Our results also raise questions regarding how metabolic disease is diagnosed and studied in humans. Currently there is a bias toward studying human and mouse metabolism in the fasted state. Equally, diagnostic tests for human metabolic disease are conducted almost exclusively in the fasted state to control for human meal choices. By extension, our data suggest that there are humans with primary adipose tissue defects who exhibit normal GTT results but become metabolically dysfunctional after eating. It is possible that a hypercaloric mixed meal tolerance test would be the most suitable way to detect such individuals. Finally, our data suggest that “murinizing” human feeding patterns may be a way to treat individuals with adipose dysfunction and storage defects.

STAR+METHODS

Detailed methods are provided in the online version of this paper and include the following:

- Experimental model and subject details
  - Animals
  - Rodent diets
  - Feeding regimes
- Method details
  - Glucose tolerance test
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  - Temperature program:
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    - Mouse Euglycaemic-hyperinsulinaemic clamp
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    - GC-MS FAME analysis
    - Lipid uptake to adipose tissue
    - LC-MS analysis
- Quantification and statistical analysis
- Data and software availability

Supplemental information includes four figures and one table and can be found with this article online at [https://doi.org/10.1016/j.celrep.2018.07.063](https://doi.org/10.1016/j.celrep.2018.07.063).

Acknowledgments

We thank Helen Westby, Sarah Grocott, Charley Beresford, Jade Bacon, Laura McKinven, and Agnes Lukasik for their excellent technical assistance. All animal work was carried out in the Disease Model Core (MRC Metabolic Diseases Unit [MRC_MC_UU_12012/5]; Wellcome Trust Strategic Award [100574/Z/12/Z]). All serum biochemistry was conducted by the Biochemistry Assay Lab (MRC Metabolic Diseases Unit [MRC_MC_UU_12012/5]). We thank the BHF (RG/12/13/29853), FIS (PI15/01934), and MRC (MC_UU_12012/2) for funding this work.

Author contributions

S.V. designed and conducted experiments and wrote the manuscript. K.P., D.H., B.J., and M.D. conducted experiments. A.K. designed and conducted experiments. J.M.M.-N. and J.M.F.-R. provided and analyzed human adipose gene expression data. A.V.-P. designed experiments and wrote the manuscript. K.P., S.V., and B.J. conducted experiments. M.R. and K.P. performed adipose and liver gene expression analysis. J.M.M.-N. provided and analyzed human feeding patterns. A.K. and K.P. performed statistical analysis.

Declaration of interests

The authors declare no competing interests.

Received: November 27, 2017
Revised: March 15, 2018
Accepted: July 18, 2018
Published: August 21, 2018

References

## STAR★METHODS

### KEY RESOURCES TABLE

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<td>1-14C Palmitate</td>
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<td>NEC075H050UC</td>
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<tr>
<td>BondElut NH2 columns</td>
<td>Agilent</td>
<td>12102014</td>
</tr>
<tr>
<td>Isolute Strong anion exchange columns, 100 mg bed, 6 ml reservoir</td>
<td>Biotage</td>
<td>500-0010-C</td>
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<tr>
<td>Formic acid</td>
<td>Sigma</td>
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<tr>
<td>Ammonium acetate</td>
<td>Sigma</td>
<td>A1542-250G</td>
</tr>
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</table>

(Continued on next page)
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed and will be fulfilled by Dr. Samuel Virtue (sv234@medschl.cam.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
PPARγ2 KO mice were generated as described previously (Medina-Gomez et al., 2005). Mice were phenotyped on a mixed C57BL/6/sv129 genetic background. Mice homozygous for a deletion in PPARγ2 (PPARγ2 /−/−) and their wild-type littermates were generated by mating heterozygous mice. All animals used were male mice. Ages are provided in figure legends. C57BL/6 wild-type mice were purchased from Charles River UK. This research has been regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB). Animals were housed in a specific pathogen free facility with 12 hour light and 12 hour dark cycles. Unless otherwise stated all animals were studied under fed conditions and at 24°C.

Rodent Diets
Mice were fed either standard breeders chow (Safe Diets, DS-105) or a 45% fat diet (HFD) (Research Diets D12451) all diets were provided ab libitum.
Feeding regimes
For the one day high-fat diet challenge mice were single housed 1 week prior to the start of the diet to permit food intake assessment. The diet commenced in a staggered manner between 10 am and midday. Mice were alternated in genotype for both feeding of the diet and also for culling. Mice were killed in the same sequence that they were fed the diet in to keep the mice as close to receiving high-fat diet for 24 hours as possible. No significant correlation between the order the mice were killed in and the levels of FFAs, insulin or TG in blood was detected for the time course of the experiment. 1 month and 1 week HFD fed animals mice were culled between 10 am and 12pm in order to maintain the animals in the same stage of the circadian cycle as the chow controls and the 1 day high-fat fed animals

Patient recruitment
Forty five subcutaneous adipose tissue (SAT) samples from morbidly obese (BMI > 35 kg/m²) participants with different degrees of insulin action (measured using hyperinsulinaemic–euglycaemic clamp) were analyzed. All these participants were of Caucasian origin, reported that their body weight had been stable for at least three months before the study and were studied in the post-absorptive state. BMI was calculated as weight (in kg) divided by height (in m) squared. Patients had no systemic disease other than obesity and all were free of any infections in the previous month before the study. Liver diseases (specifically tumoral disease and hepatitis C virus infection) and thyroid dysfunction were specifically excluded by biochemical work-up. All these participants were recruited at the Endocrinology Service of the Hospital of Girona ‘Dr Josep Trueta’. All participants gave written informed consent, validated and approved by the Ethics Committee of the Hospital of Girona ‘Dr Josep Trueta’, after the purpose of the study was explained to them.

METHOD DETAILS

Glucose tolerance test
For fasting glucose tolerance tests mice were fasted overnight from 4 pm until 9 am the next day and then allowed to acclimatise to the procedural room for 1 hour. For fed glucose tolerance tests mice were allowed to accclimatise to the procedural room for one hour between 9 and 10 am. Glucose was administered at 1 g/kg IP. A fixed dose of glucose was given to all mice in the study group based on the average weight of the group. Fed glucose tolerance tests were performed at 10 am in the morning. Blood samples were collected at 0, 10, 20, 30, 60, 90 and 120 minutes.

For stable isotope GTTs mice were injected with a 50:50 mix of D2-6,6 Glucose and D2-2 Glucose (Goss Scientific, UK). Blood was collected on blood spot cards. A 6 mm punch was removed from each blood spot card and extracted by wetting with 40 ul of water followed by addition of 400 ul of ethanol. Samples were shaken for 45 minutes at room temperature before undergoing centrifugation at 16,000 g. ETOH extracts were dried under vacuum at ambient temperature using a Speed Vac. Extracts underwent aldonitrile pentaacetate derivitisation as follows: Dried extracts were resuspended in 50 ul of pyridine containing 2% w/v hydroxylamine. Samples were heated for 45 minutes at 90 °C then allowed to cool. After cooling 100 ul of acetic anhydride was added and samples were heated for 30 minutes at 60 °C for 30 minutes. Samples were allowed to cool and then dried under nitrogen at 60 °C. Dried samples were dissolved in 200 ul of ethyl acetate, centrifuged at 16,000 g for 1 minute and the top 150 ul of supernatant was transferred to an autosampler vial containing a 300 ul glass insert. Gas-Chromatography Mass spectrometry analysis was performed using an Agilent 7890B GC and an Agilent 5977A MSD using a 0.25 uM x 30 M DB-5 (Cat: 122-5031, Agilent) column. The GC-Conditions were as follows:

Inlet conditions

<table>
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</thead>
<tbody>
<tr>
<td>Inlet temperature</td>
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<tr>
<td>Split</td>
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</tr>
<tr>
<td>Inlet liner</td>
<td>Liner, ultra inert, splitless, single taper, glasswool (cat#6322616) (Agilent Technologies)</td>
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<tr>
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Temperature Program:

<table>
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</thead>
<tbody>
<tr>
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<tr>
<td>20 °C/min until</td>
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<tr>
<td>280 °C Hold</td>
<td>3 minutes</td>
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MSD conditions:

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</thead>
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<tr>
<td>MS Source temperature</td>
<td>230 °C</td>
</tr>
<tr>
<td>MS Quad temperature</td>
<td>150 °C</td>
</tr>
</tbody>
</table>
Analysis was performed using MS Quantitative analysis. Ions analyzed were 217 (M0), 218 (M1), 219 (M2), 220 (M3) and 221 (M4). To determine the relative ratio of endogenous to exogenous glucose blood samples were compared to the glucose composition of mouse blood from animals uninjected with labeled glucose and a sample of D2-6,6 glucose (10 ul, 10 mM) that was dried onto a blood spot card and extracted as described above. The ion pattern of each blood sample was compared to the tracer and endogenous blood glucose. To determine the relative contribution of endogenous and tracer glucose we defined 5 equations for each ion as follows:

Equation System 1:

\[
M0_{\text{calc}} = x \cdot M0_{\text{Endo}} + (1-x) \cdot M0_{\text{Exo}} \\
M1_{\text{calc}} = x \cdot M1_{\text{Endo}} + (1-x) \cdot M1_{\text{Exo}} \\
M2_{\text{calc}} = x \cdot M2_{\text{Endo}} + (1-x) \cdot M2_{\text{Exo}} \\
M3_{\text{calc}} = x \cdot M3_{\text{Endo}} + (1-x) \cdot M3_{\text{Exo}} \\
M4_{\text{calc}} = x \cdot M4_{\text{Endo}} + (1-x) \cdot M4_{\text{Exo}}
\]

Where \(x\) represents the Fractional concentration of the endogenous glucose in the blood and \(1-x\) the Fractional concentration of exogenous glucose in the blood.

We defined a further 5 equations:

Equation System 2:

\[
M0_{\text{obs}} = M0_{\text{calc}} \\
M1_{\text{obs}} = M1_{\text{calc}} \\
M2_{\text{obs}} = M2_{\text{calc}} \\
M3_{\text{obs}} = M3_{\text{calc}} \\
M4_{\text{obs}} = M4_{\text{calc}}
\]

We then used the Solver function of Excel to minimize the sum of the squares of the result of the 5 equations by changing the value of \(x\) in equation system 1 using the GRG Non-Linear Engine.

The solution for \(x\) defined the fractional concentration of endogenous glucose and \(1-x\) the fractional concentration of D2-6,6 glucose. As D2-6,6 glucose represented 50% of the glucose injected in the GTT we multiplied the fractional concentration of D2-6,6 glucose by 2 to get the fractional concentration of the exogenous glucose and multiplied it by the observed blood concentration of glucose to get the concentration of exogenous glucose in mMol. By subtraction the exogenous glucose from the mouse’s total blood glucose concentration we obtained the endogenous glucose concentration.

**Mouse Euglycaemic-hyperinsulinaemic clamp**

Animals were fasted from 4 pm until 8 am the next morning. Animals were anaesthetised with 11 \(\mu\)l per gram of body weight using a combination of the following drugs at stated concentrations fentanyl (0.03125 mg/ml), midazolam (0.625 mg/ml), and acepromazine (0.625 mg/ml). Once unconscious mice were placed on temperature controlled heating pads (Harvard Apparatus) and core body temperature was maintained at 37°C. Mice underwent a basal infusion period. The basal infusate comprised of 3-3H glucose (0.23 MBq/ml) and 3 mg/ml sodium citrate in 0.9% saline. The basal infusion rate was 100 ul/hour and was conducted for 90 minutes. For the clamp phase the basal infusate was stopped and the hyperinsulinaemic infusion was started. The hyperinsulinaemic infusate comprised of 0.11 mU per ml of insulin, 0.231 MBq/ml of 3-3H glucose, 3 mg/ml sodium citrate, 2% FFA-free low-endotoxin BSA in 0.9% saline. The hyperinsulinaemic infusion was initiated with a bolus of 30 ul of infusate followed by 100 ul/hour. A variable rate infusion of glucose was then started using 12.5% glucose in 0.9% saline with 3 mg/ml sodium citrate. Blood glucose was measured every 5 minutes and glucose infusion rate was adjusted to clamp mice to their basal glucose levels. The hyperinsulinaemic infusion was continued for at least 90 minutes, or if mice had not reached steady state until steady state was achieved. Steady state was defined as mice exhibiting blood glucoses that were ± 0.5 mMol of their starting blood glucose and having a stable glucose infusion rate for three consecutive measurements. Blood samples were collected for the determination of FFA, Insulin and serum specific activity at the end of the basal and clamped states.

**Mouse 2-Deoxyglucose uptake**

Mice were fed a high-fat diet for 1 day, with mice placed on the diet between 8 and 9 am. The following day mice were anaesthetised in the sequence they were anaesthetised between 8 and 9 am. The anesthetic was 11 \(\mu\)l per gram of body weight using a combination of the following drugs at stated concentrations fentanyl (0.03125 mg/ml), midazolam (0.625 mg/ml), and acepromazine (0.625 mg/ml). Mice were injected IV with 0.046 MBq of 14C 2-Deoxyglucose and blood glucose levels were measured and serum samples collected at 10, 20 and 30 minutes post injection in order to determine the specific activity of glucose in the blood. Mice were killed 30 minutes after injection with 14C-2-Deoxyglucose and tissues were harvested.

Phospho deoxyglucose was separated from deoxyglucose using solid-phase extraction with strong anion exchange columns. Tissues were weighed (~100 mg of WAT and ~50 mg of BAT) and homogenized in 1 mL of distilled water in glass tubes. The homogenates were heated to 95°C for 10 minutes before cooling to room temperature. Homogenates were then transferred to eppendorf tubes and centrifuged at 16,000 G and 66 ul of homogenate was taken for scintillation counting. SAX-SPE columns were conditioned
Mice were fasted overnight from 4 pm until 9 am the next day. Mice were gavaged with 200 mL of olive oil (Heritage, NISA, UK). Blood samples were collected at 0, 1, 2, 4, and 8 hours post-gavage for determination of TG and FFA levels. See Serum biochemistry for details of TG and FFA measurement.

Liver fat %

100-200 mg of liver was weighed and lipids extracted according to the Folch extraction procedure (Folch et al., 1957). Briefly, 100 mg of liver was homogenized in 1 mL volumes (v/v) of 2:1 chloroform methanol using a MP Biomedical Fast Prep. Homogenized samples were centrifuged (16,000 G for 10 minutes) and the supernatant transferred to a clean Eppendorf tube. 200 ul of water was added and samples vortexed for 2 minutes. Samples were centrifuged (16,000 G for 10 minutes) and the lower organic phase was collected into a pre-weighed Eppendorf tube. A second round of extraction was carried out by adding 700 ul of chloroform to the sample and vortexing (2 minutes) and centrifuging (16,000 G for 10 minutes). The lower organic phase was collected again and combined with the previous extract. The extract was then dried under nitrogen. The weight of the tube and extracted lipid was measured and the weight of the empty tube subtracted to give the quantity of lipid in the sample. The liver fat extract was expressed as a % of wet liver weight.

Serum Biochemistry

Triglycerides were measured on the Dimension RXL analyzer (Siemens Healthcare). Free Fatty Acids were measured using the Roche Free Fatty Acid Kit (half-micro test) (kit code 11383175001). Insulin, was measured using electrochemical luminescence immunoassay on the MesoScale Discovery immunoassay platform.

RNA extraction and real time PCR

RNA was extracted using STAT-60 (AMS biotech) according to manufacturer’s procedures. Reverse transcription (500 ng of total RNA) was performed using random hexamers using the Reverse Transcriptase System (Promega) according to manufacturer’s instructions. Real-time PCR was carried out in 13 ul reactions using TaqMan or Sybr Green reagents and performed in a 384 well plate on an ABI7900 real-time PCR machine using default thermal cyclor conditions. For PCR primer sequences see Table S1.

Western Blotting

Protein was extracted from tissues using a modified RIPA buffer (50 mM Tris HCL pH 8, 150 mM NaCl, 1% NP-40, 0.5% Sodium Deoxycholate, 0.1% SDS) and quantified by the BioRad DC protein assay. Protein was heated with loading dye containing DTT and heated to 95°C for 5 minutes to denature proteins before loading. 10 pg per well of protein was subjected to SDS-PAGE in a 4%–12% gradient gel using the Novex NuPage midi system (Life Technologies) and transferred using the iBlot transfer system and reagents (Life Technologies) for 7 minutes. Membranes were probed for: pHSL S660 (Cat: 4126 Cell Signaling, USA) total HSL (Cat: 4107 Cell Signaling, USA), Phospho-p44/42 MAPK (Cat: 4376 Cell Signaling, USA), total p44/42 MAPK (Cat: 4695 Cell Signaling, USA), Phospho AKT (Cat: 4060 Cell Signaling, USA), total-AKT (Cat: 2920 Cell Signaling, USA) and B-actin (Cat: 8226 Abcam, UK).

Histological analysis of white adipose tissue

Adipose tissue was fixed in formalin. Tissues were embedded in paraffin and sections were cut at a thickness of 100 μM. Slides were imaged using an automated slide scanning microscope (Axioscan Z1 using a Hamamatsu orca flash 4.0 V3 camera) using a 20x objective with a numerical aperture of 0.8. Adipocytes size was quantified using Halo (Indica Labs inc) utilizing the Vacuole module v.1.4. The cut off point for minimum vacuole diameter was set at 15 and the maximum at 150 μM. Adipocyte, blood vessels and slide background were determined using the tissue classifier method, only adipocytes were quantified.
GC-MS FAME analysis

Lipid extraction and analysis were described in detail previously (Yew Tan et al., 2015). Briefly, lipids were extracted by the Folch extraction procedure. Lipids were esterified to form methyl esters using BF3 methanol. Methyl esters were analyzed by Gas-chromatography Mass-Spectrometry. The identity of species was determined by retention time and mass spectra and compared to a food industry standard (Thames Restek, UK). Lipid species were quantified based on integrated peak area.

Lipid uptake to adipose tissue

Mice were caged as described above. 30 minutes from the end of the clamp a bolus of 0.2 MBQ of 1-14C palmitate conjugated to 2% BSA in PBS was injected IV. After 30 minutes serum and adipose tissue was collected. Serum DPMs were measured directly. Adipose tissue was weighed and lipids extracted using the Folch method (see “Liver fat%” above). Lipids were separated into Neutral Lipid, Phospholipid and FFA fractions by solid phase extraction. Solid phase extraction was performed by resuspending the adipose tissue extract in 1 mL of dry chloroform. Of this 1 mL 100 µl was reserved for scintillation counting and 900 µl was applied to the SPE column and the flow through collected. Two further washes of 1 mL of dry chloroform were performed and combined with the initial flow through; this represented the neutral lipid fraction. Phospholipids were then eluted from the columns into a fresh tube using 2 mL of Chloroform:Methanol 60:40. The NEFA fraction was eluted in 2.0ml chloroform/methanol/glacial acetic acid (100:2:2). All SPE steps were performed using a vacuum manifold. Unlike many SPE lipid extraction protocols, no activation of the columns was required and between collection of fractions the bed was dried under vacuum for 2-3 s to minimize carry over between fractions.

LC-MS analysis

Sample extraction

The pre-weighed muscle tissue samples (~10-20 mg) were transferred to a 2 mL Eppendorf screw cap tubes. A single 5 mm stainless steel ball bearing and 400 µL of the chloroform:methanol mix solution (2:1, respectively) were added to the tissue samples, followed by a 3 s cycle of vigorous vortexing. The tissues were then homogenized in the extraction solvent using a Bioprep-24-1004 homogenizer (Allsheng, Hangzhou City, China) run at speed; 6 m/s, time; 30 s for 2 cycles. Following the homogenization, 150 µL of the stable isotope labeled lipid internal standard mix was added along with 10 µL the stable isotope 5-hydroxytryptamine-d4 acylcarnitine internal marker. The mixtures was then thoroughly vortexed before the addition of 600 µL of the chloroform:methanol mix solution (2:1, respectively). The samples were homogenized again (speed; 6 m/s, time; 30 s for 2 cycles). To the homogenates, 400 µL of HPLC-water was added with an additional cycle of vortexing. The samples were then centrifuged (5 mins at ~21,000 g) to produce aqueous and organic biphasic extracts. The two phases were then separated by pipetting each into separate 2 mL Eppendorf screw cap vials making sure not to break up the undissolved protein pellet. To perform a double extraction, 1 mL of the chloroform:methanol mix solution (2:1, respectively) was added to each sample, then vortexed thoroughly. The samples were then homogenized on the tissue homogenizer (speed; 6 m/s, time; 30 s for 2 cycles). Then 400 µL of HPLC-water was added to each sample, vortexed and homogenized on the tissue homogenizer (speed; 6 m/s, time; 30 s for 2 cycles). The samples were then centrifuged (5 mins at ~21,000 g) to produce the biphasic extracts, followed by phase separation into the corresponding 2 mL Eppendorf screw cap vials containing the first extracts.

Acylcarnitine sample preparation

Half of the corresponding organic fractions and half the aqueous fraction were mixed with each other into separate 2 mL Eppendorf screw cap vials. The mixed extracts were dried completely under a gentle stream of oxygen free nitrogen heated to 45°C. To the dried extracts, 200 µL of water:acetonitrile (4:1, respectively) was added to reconstitute the acylcarnitine species. The samples were then vortexed thoroughly. The samples were then transferred into 2 mL amber glass vials containing a 300 µL vial-insert which were then analyzed by liquid chromatography with mass spectrometry detection.

Acylcarnitine LC-MS

Chromatographic separation was achieved using an ACE Excel 2 C18-PFP (150mm * 2.1mm, 2 µm) LC-column with a Shimadzu UPLC system (Shimadzu UK Limited, Wolverton, Milton Keynes). The column was maintained at 55°C with a flow rate of 0.5 mL/min. A binary mobile phase system was used with mobile phase A; water (with 0.1% formic acid), and mobile phase B; acetonitrile (with 0.1% formic acid). The gradient profile was as follows; at 0 minutes_0% mobile phase B, at 0.5 minutes_100% mobile phase B, at 5.5 minutes_100% mobile phase B, at 5.5 minutes_0% mobile phase B, at 7 minutes_0% mobile phase B. Mass spectrometry detection was performed on a Thermo Exactive orbitrap mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) operating in positive ion mode. A heated electrospray source was used; the sheath gas was set to 40 (arbitrary units), the aux gas set to 15 (arbitrary units) and the capillary temperature set to 250°C. The instrument was operated in full scan mode from m/z 75–100 Da. Acylcarnitine species were identified by detecting a signal peak for the corresponding accurate mass at the correct retention time. Signals were normalized to the dried tissue mass (dried protein pellet left after the extraction), their final semiquantitative concentrations were determined by comparing the acylcarnitine species intensity to the internal marker intensity.

Lipid sample preparation, 50 µL of the organic fractions were transferred into 2 mL amber glass vials containing a 300 µL vial-insert. The samples were then dried completely under a gentle stream of oxygen free nitrogen at 45°C. Then, 90 µL of isopropanol:acetoni trile (4:1, respectively) was added to reconstitute the lipid species. The samples were then vortexed thoroughly before being analyzed by liquid chromatography with mass spectrometry detection.

Lipid LC-MS, Chromatographic separation was achieved using a Waters Acquity UPLC CSH C18 (50mm * 2.1mm, 1.7 µm) LC-column with a Shimadzu UPLC system (Shimadzu UK Limited, Wolverton, Milton Keynes). The column was maintained at 55°C with a
flow rate of 0.5 mL/min. A binary mobile phase system was used with mobile phase A: acetonitrile:water mix (6:4, respectively, with 10 mM ammonium formate), and mobile phase B; isopropanol:acetonitrile mix (9:1, respectively, with 10 mM ammonium formate). The gradient profile was as follows; at 0 minutes, 40% mobile phase B, at 0.4 minutes, 43% mobile phase B, at 0.45 minutes, 50% mobile phase B, at 2.4 minutes, 54% mobile phase B, at 2.45 minutes, 70% mobile phase B, at 7 minutes, 99% mobile phase B, at 8 minutes, 99% mobile phase B, at 8.3 minutes, 40% mobile phase B, at 10 minutes, 40% mobile phase B. Mass spectrometry detection was performed on a Thermo Exactive orbitrap mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) operating in positive ion and negative ion continuous switching mode. Heated electrospray source was used; the sheath gas was set to 40 (arbitrary units), the aux gas set to 15 (arbitrary units) and the capillary temperature set to 300 °C. The instrument was operated in full scan mode from m/z 150–1200 Da. Lipid species were identified by detecting a signal peak for the corresponding accurate mass at the correct retention time. Signals were normalized to the dried tissue mass (dried protein pellet left after the extraction), their final semi-quantitative concentrations were determined by comparing the lipid species intensity to the appropriate stable isotope labeled lipid internal standard.

**Human Adipose tissue handling**

Adipose tissue samples were obtained from SAT depots during elective surgical procedures (cholecystectomy, abdominal hernia surgery and gastric bypass surgery). Samples of adipose tissue were immediately transported to the laboratory (5-10 min). The handling of tissue was carried out under strictly aseptic conditions. Adipose tissue samples were washed in PBS, cut with forceps and scalpels into small pieces (100 mg) and immediately flash-frozen in liquid nitrogen before being stored at −80°C.

**Hyperinsulinemic-euglycemic clamp in humans**

After an overnight fast, two catheters were inserted into an antecubital vein, one for each arm, used to administer constant infusions of glucose and insulin, and to obtain arterialized venous blood samples. A 2-h hyperinsulinemic-euglycemic clamp was initiated by a two-step primed infusion of insulin (80mU/m²/min for 5 min, 60mU/m²/min for 5 min) immediately followed by a continuous infusion of insulin at a rate of 40mU/m²/min (regular insulin (Actrapid, Novo Nordisk, NJ)). Glucose infusion began at minute 4 at an initial perfusion rate of 0.011 mmol/kg/min being then adjusted to maintain plasma glucose concentration at 4.9 – 5.5 mmol/l. Blood samples were collected every 5 min for determination of plasma glucose and insulin. Insulin sensitivity was assessed as the mean glucose infusion rate during the last 40 min. In the stationary equilibrium, the amount of glucose administered (M) equals the glucose taken by the body tissues and is a measure of overall insulin sensitivity.

**RNA expression for human SAT**

RNA purification was performed using an RNasey Lipid Tissue Mini Kit (Qiagen, Izasa, Barcelona, Spain) and the integrity was checked using an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Gene expression was assessed by real-time PCR using a LightCycler 480 Real-Time PCR System (Roche Diagnostics, Barcelona, Spain), using TaqMan and SYBRgreen technology. The RT-PCR reaction was performed in a final volume of 12 μL. The cycle program consisted of an initial denaturing of 10 min at 95 °C then 40 cycles of 15 s denaturizing phase at 95 °C and 1 min annealing and extension phase at 60 °C. A threshold cycle (Ct value) was obtained for each amplification curve and a ΔCt value was first calculated by subtracting the Ct value for human cyclophilin A (PPIA) RNA from the Ct value for each sample. Fold changes compared with the endogenous control were then determined by calculating 2ΔΔCt, so gene expression results are expressed as expression ratio relative to PPIA gene expression according to manufacturers’ guidelines. The commercially available and pre-validated TaqMan® primer/probe sets used were as follows: Peptidylprolyl isomerase A (cyclophilin A) (4335763, PPIA as endogenous control), insulin receptor substrate 1 (IRS1, Hs00178563_m1), solute carrier family 2 member 4 (SLC2A4 or GLUT4, Hs00168966_m1). Human PPARγ1 [forward: 5’-GGGCAGAGTTGAAAGAG-3’ and reverse: 5’-GGAGAGATCCAGGGAGTGAG-3’] and PPARγ2 [forward: 5’-AAACCCCTATCCATGCTTTATG-3’ and reverse: 5’-TGCTAACCATGGTCTTGTGTG-3’] were measured using SYBR Green technology.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All statistics were performed using SPSS 25. Data points were excluded if they exhibit a value of more than two standard deviations from the mean. For all metabolic tests animals were randomly ordered into metabolic chambers (calorimetry) or to order in which experiments were conducted (GTG, Euglycaemic-hyperinsulinaemic clamps). Statistical significant was set at a p value of < 0.05. Specific tests are detailed in the figure legends.

**DATA AND SOFTWARE AVAILABILITY**

https://data.mendeley.com/datasets/whn4s7nyw/draft?a=22693287-b601-457c-85a9-9903c6d88639