INFLAMED FAT: immune modulation of adipose tissue and lipid metabolism

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Chapter 6

GPR120 as a novel target to increase lipid oxidation and reduce obesity

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ABSTRACT

The free fatty acid receptor GPR120 is highly expressed in white adipose tissue and brown adipose tissue (BAT), both of which play a major role in triglyceride metabolism. However, the effects of GPR120 on lipid metabolism and substrate utilization have not been studied to date. The aim of our current study was to assess the role of GPR120 in lipid metabolism. To this end, GPR120$^{-/-}$ and wild-type mice (C57BL/6 background) received a high-fat diet (HFD) for 8 weeks. Body composition was monitored by EchoMRI and fully automated metabolic cages were used to measure energy expenditure and substrate utilization. GPR120$^{-/-}$ mice had higher fat mass (+25%, $p=0.05$), lower physical activity (-49%, $p<0.05$) and lower energy expenditure during the dark phase (-5%, $p<0.05$), albeit that substrate utilization was not different (similar respiratory exchange ratio) compared to wild-type mice. GPR120 deficiency reduced the expression of *Ucp1*, *Prdm16* and *Pparα* in BAT (-38 to -59%, $p<0.05$) without reducing uptake of fatty acids derived from intravenously injected lipoprotein-like particles labeled with glycerol tri[^{3}H]oleate ([^{3}H]TO) by BAT. When wild-type mice were fed a HFD for 6 weeks and orally treated with the GPR120 agonist TUG891 (10 mg/kg daily) during the last 3 weeks of HFD feeding, TUG891 reduced fat mass after 2.5 weeks (-40%, $p<0.05$). To assess acute effects of the GPR120 agonist, wild-type mice on a chow diet were treated with i.p. TUG891 (35 mg/kg daily) for 5 days. Although i.p. TUG891 treatment did not increase total energy expenditure, the GPR120 agonist acutely increased fat oxidation (+331%, $p<0.01$) while reducing glucose oxidation (-18%, $p<0.05$), which was accompanied by a tendency for reduced fat mass already after 5 days (-19%, $p=0.06$). In conclusion, GPR120 deficiency increases fat mass without evident effects on triglyceride-rich lipoprotein turnover and GPR120 agonism reduces fat mass accompanied by increased fat oxidation. Therefore, stimulation of GPR120 holds therapeutic potential to combat obesity.

INTRODUCTION

G protein-coupled receptors (GPCRs) represent the largest family of transmembrane signaling receptors (1). A subclass of the GPCR family specifically binds free fatty acids (FFAs), which can act as signaling molecules to regulate various physiological processes. GPR120, also known as free fatty acid receptor 4 (FFAR4), is activated by medium and long chain fatty acids (2). Recent studies have revealed an important role for GPR120 in immune regulation, hormonal secretion and energy metabolism. For example, several studies showed that GPR120 mediates anti-inflammatory actions of ω-3 fatty acids (3-5). In white adipose tissue (WAT), GPR120 plays a role in adipocyte differentiation (6) and enhances glucose uptake, which contributes to improved insulin sensitivity (3). GPR120 deficiency leads to obesity, glucose intolerance and hepatic steatosis in mice fed a high-fat diet (7). In humans, individuals carrying a mutation associated with decreased GPR120 signaling have an increased risk of obesity (7). Together, these negative effects of reduced GPR120 signaling have led to high interest in the development of pharmacological compounds to activate this receptor (8, 9).

GPR120 is expressed in both WAT and brown adipose tissue (BAT). WAT is the most abundant adipose tissue type, found throughout the body in different subcutaneous and visceral depots (10). WAT is a major participant in energy regulation of the body, by storing excess ingested glucose and fatty acids in the form of triglycerides within adipocytes and by releasing fatty acids to meet the energy needs of other organs (11). In contrast to WAT, BAT combusts fatty acids to generate heat for maintenance of body temperature, defined as non-shivering thermogenesis. Brown adipocytes are smaller than white adipocytes, contain many mitochondria and typically contain multiple small lipid droplets (10). Thermogenesis is dependent on the presence of uncoupling protein 1 (UCP1), which is present in mitochondria and "uncouples" electron transport from ATP synthesis. As a consequence, heat is generated instead of ATP (12, 13). Interestingly, GPR120 is highly expressed in BAT and cold exposure even further increases its expression in BAT and subcutaneous (s)WAT (14), indicating a role for GPR120 in thermogenesis. Also, GPR120 expression increases in WAT of mice on a high-fat diet (6). Collectively, these data suggest GPR120 plays a role in both BAT and WAT physiology.

Although previous studies have focused on the metabolic effects of GPR120 signaling, most focused on the effects of GPR120 on gut hormone secretion and glucose metabolism. The aim of our current study was to assess the role of GPR120 in lipid metabolism and the therapeutic potential of a selective GPR120 agonist. To this end, we studied lipid metabolism in GPR120$^{-/-}$ mice and treated wild-type mice with the GPR120 agonist TUG891. We found that GPR120 deficiency increases fat mass, reduces physical activity and reduces the expression of BAT-specific genes, but does not have pronounced effects on lipid metabolism. Interestingly, we found that specific stimulation of GPR120 with TUG891 increases fat oxidation and reduces fat mass. Taken together, our data indicate that GPR120 deficiency increases fat mass without evident effects on triglyceride-rich

lipoprotein turnover and GPR120 agonism reduces fat mass accompanied by increased fat oxidation. Therefore, GPR120 may be a promising target to reduce obesity.

MATERIALS AND METHODS

Animals, diet and treatment

All mice were approximately 12 weeks of age at the start of each experiment and individually housed under standard conditions with a 12:12 h light-dark cycle (from 7:00 h to 19:00 h) and free access to food and water. To assess the effect of GPR120 deficiency on the development of obesity, male GPR120$^{-/-}$ mice and wild-type mice on a C57Bl/6 background were acquired from Taconic Biosciences. Mice received a high-fat diet (HFD; 45% kcal from lard fat, Research Diets) for 8 weeks, upon which they were sacrificed by cervical dislocation. Two wild-type mice were excluded due to severe infection during the study. One GPR120$^{-/-}$ mouse was excluded because of unexplained weight loss after 4 weeks of HFD.

To investigate the effect of oral treatment with a GPR120 agonist on obesity, male C57Bl6/J mice (Charles River Laboratories) were fed a HFD for 3 weeks before initiation of treatment with TUG891 at 12 weeks of age. TUG891 was synthesized as described previously (15). Mice were treated with vehicle or TUG891 (10 mg/kg body weight, dissolved in 10% v/v DMSO, 10% w/v cremophor and 5% w/v mannitol in water, all from Sigma-Aldrich) by oral gavages daily at 15:00 h. Treatment lasted for 3 weeks, upon which the mice were sacrificed by cervical dislocation.

The acute effects of TUG891 on energy expenditure were assessed in male C57Bl6/J mice. First, mice were i.p. injected twice daily at 9:00 h and 16:00 h with vehicle or increasing doses of TUG891 (dissolved in 10% v/v DMSO in PBS) ranging from 15-50 mg/kg body weight as indicated over a period of 5 days. Thereafter, mice were i.p. injected once daily at 17:00 h with 35 mg/kg body weight TUG891 for 5 consecutive days. Mouse experiments were performed in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and had received approval from the University Ethical Review Board (Leiden University Medical Center, The Netherlands).

Food intake, body weight, and body composition

At indicated time points, food intake and body weight were measured with a scale, and body composition was measured using an EchoMRI-100 analyzer (EchoMRI, TX, USA).

Energy metabolism

GPR120$^{-/-}$ and wild-type control mice were housed in fully automatic metabolic cages (LabMaster System; TSE Systems) in the second week of HFD-feeding. Wild-type mice that received i.p. treatment with TUG891 were housed in metabolic cages during the entire treatment period. Metabolic cages measured oxygen uptake (V_{O2}) and carbon dioxide production (V_{CO2}). Respiratory exchange ratio (RER), glucose oxidation and fat oxidation were calculated from V_{O2} and V_{CO2} as described previously (16). Total energy expenditure was

calculated from V_{O2} and V_{CO2} using the Weir equation (17). Physical activity was measured with infrared sensor frames.

Histology of WAT and BAT

Subcutaneous WAT and interscapular BAT (iBAT) were removed, fixed in 4% paraformaldehyde, dehydrated in 70% EtOH, and embedded in paraffin. Hematoxylin-eosin (HE) stainings were performed on sections (5 µm) using standard protocols. The cell size of adipocytes in WAT and the area of intracellular lipid vacuoles in BAT were quantified using ImageJ software.

RNA purification and quantitative RT-PCR

RNA was extracted from snap-frozen iBAT using Tripure RNA Isolation reagent (Roche) according to manufacturer's instructions. RNA concentrations were measured using NanoDrop and RNA was reverse transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (Promega) for quantitative RT-PCR (qRT-PCR) to produce cDNA. Expression levels of genes were determined by qRT-PCR, using gene-specific primers (Table 1) and SYBR green supermix (Biorad). mRNA expression was normalized to *B2m* and *Gapdh* mRNA content and expressed as fold change compared with control mice using the ΔΔCT method.

Table 1. *Primer sequences of forward and reverse primers (5' → 3').*

Gene	Forward primer	Reverse primer
Angptl4	GGAAAGAGGCTTCCCAAGAT	TCCCAGGACTGGTTGAAGTC
Ap2	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
B2m	TGACCGGCTTGTATGCTATC	CAGTGTGAGCCAGGATATAG
Cd36	GCAAAGAACAGCAGCAAAATC	CAGTGAAGGCTCAAAGATGG
Cpt1a	GAGACTTCCAACGCATGACA	ATGGGTTGGGGTGATGTAGA
Elovl3	GGATGACGCCGTAGTCAGTA	GACAGAATGGACGCCAAAGT
Gapdh	GGGGCTGGCATTGCTCTCAA	TTGCTCAGTGTCCTTGCTGGGG
Glut1	AGCATCTTCGAGAAGGCAGG	ACAACAAACAGCGACACCAC
Glut4	CAGCGCCTGAGTCTTTTCTT	GGCATTGATAACCCCAATGT
Gpr120	ACATTGGATTGGCCCAACCGCA	TCCGCGATGCTTTCGTGATCTGT
Lpl	CCCTAAGGACCCCTGAAGAC	GGCCCGATACAACCAGTCTA
Pgc1a	TGCTAGCGGTTCTCACAGAG	AGTGCTAAGACCGCTGCATT
Ppara	ATGCCAGTACTGCCGTTTTC	GGCCTTGACCTTGTTCATGT
Pparγ	GTGCCAGTTTCGATCCGTAGA	GGCCAGCATCGTGTAGATGA
Prdm16	ACTTTGGATGGGAGCAGATG	CTCCAGGCTCGATGTCCTTA
Ucp1	TCAGGATTGGCCTCTACGAC	TGCATTCTGACCTTCACGAC
Vegfα	GGAGATCCTTCGAGGAGCACTT	GGCGATTTAGCAGCAGATATAAGAA

Plasma lipids and glucose

At the indicated time points, 4 h-fasted (from 8:00 to 12:00) blood samples were collected from GPR120$^{-/-}$ and wild-type control mice and 6 h-fasted (from 8:00 to 14:00) blood samples were collected from mice that were orally treated with TUG891 or vehicle. This was done by tail vein bleeding into chilled capillaries that were coated with paraoxon (Sigma-Aldrich) to prevent ongoing lipolysis (18). Isolated plasma was assayed for triglycerides, glucose, and free fatty acids. Triglyceride and glucose levels were measured by commercially available enzymatic kits (from Roche Diagnostics and Instruchemie, respectively). Free fatty acids were measured using the NEFA C kit (Wako Diagnostics; Instruchemie).

Olive oil tolerance test

Postprandial triglyceride response was measured in GPR120$^{-/-}$ and wild-type control mice after 6 weeks of HFD. Animals were fasted for 4 h (from 7:00 h to 11:00 h) and a basal blood sample was drawn before an intragastric load of 200 µL olive oil (Carbonell, Traditional, Spain) was given. Blood samples were drawn 1, 2, 4 and 8 h after the bolus via tail vein bleeding and plasma triglyceride and free fatty acid levels were measured as described above.

In vivo clearance of lipoprotein-like emulsion particles and glucose

Lipoprotein-like emulsion particles were prepared from 100 mg of total lipid, including glycerol trioleate (triolein; TO; 70 mg), egg yolk phosphatidylcholine (22.7 mg), lysophosphatidylcholine (2.3 mg), cholesteryl oleate (3.0 mg) and cholesterol (2.0 mg), with addition of [^{3}H]TO (3.7 MBq). Lipids were sonicated and resulting particles fractionated by sequential density gradient ultracentrifugation steps (19). The emulsion fraction containing lipoprotein-like particles of 80 nm was isolated and mixed with 2-[1-^{14}C]deoxy-D-glucose ([^{14}C]DG; 4:1 ratio, based on radioactive count). Mice were fasted for 6 h (from 7:00 h to 13:00 h) and injected with 200 µL of [^{14}C]DG and [^{3}H]TO-labeled lipoprotein-like particles (1.0 mg triglycerides per mouse) via the tail vein. Blood samples were drawn from the tail vein at 2, 5, 10 and 15 minutes after injection to determine the plasma decay of [^{3}H]TO and [^{14}C]DG. After 15 min, mice were sacrificed by cervical dislocation and perfused with ice-cold PBS through the heart. Organs were harvested and weighed, dissolved in Tissue Solubilizer (Amersham Biosciences) overnight at 56°C and analyzed for ^{3}H- and ^{14}C-activity.

Statistical Analysis

All data are expressed as means ± SEM. Groups were compared with a two-tailed unpaired Student's t-test or a two-way ANOVA for repeated measurements, as indicated. Probability values less than 0.05 were considered statistically significant.

RESULTS

GPR120 deficiency increases HFD-induced fat accumulation and reduces physical activity

To investigate the consequences of GPR120 deficiency on the development of obesity, GPR120$^{-/-}$ and wild-type control mice were fed a HFD for 8 weeks. Body weight (**Fig. 1A**) was comparable between the groups throughout the study, although GPR120$^{-/-}$ mice exhibited less lean mass (23.5 *vs.* 25.7 g, $p<0.05$; **Fig. 1B**) and more fat mass (2.8 *vs.* 1.0 g, $p<0.01$; **Fig. 1C**) at the beginning of the HFD. The difference in lean mass disappeared while the difference in fat mass in GPR120$^{-/-}$ enlarged during the time course of HFD feeding. The increased fat mass was mainly due to an increase in the sWAT depot, while gWAT mass was comparable between GPR120$^{-/-}$ and control mice after 8 weeks of HFD (**Fig. 1D**). The iBAT depot was also larger but this does not contribute substantially to total body fat or weight. The increase in fat mass could not be explained by differences in food intake as cumulative food intake was lower in GPR120$^{-/-}$ mice compared to controls (**Fig. 1E**). Instead, total energy expenditure was lower in GPR120$^{-/-}$ animals during the dark phase (**Fig. 1F, G**). The latter could possibly be due to the markedly lower physical activity levels observed in GPR120$^{-/-}$ mice as compared to controls (**Fig. 1H, I**). Despite a lower energy expenditure, GPR120 deficiency did not affect substrate utilization, as the respiratory exchange ratio (RER) was similar in GPR120$^{-/-}$ and control mice (**Fig. 1J**).

GPR120 deficiency reduces BAT-specific gene expression

To further investigate the cause of the increased fat mass observed in GPR120$^{-/-}$ mice, we more closely assessed the sWAT and iBAT depots. In line with the increased sWAT depot weight, the average size of adipocytes in the sWAT depot of GPR120$^{-/-}$ mice tended to be larger than in controls (**Fig. 2A, B**). Albeit that lipid droplet content in iBAT was not significantly higher in GPR120$^{-/-}$ mice (**Fig. 2C, D**), markers of active BAT including *Ucp1*, *Elovl3*, *Prdm16*, *Ppara* and *Vegf* were all lower in GPR120$^{-/-}$ mice compared to controls (**Fig. 2E**). Of the markers involved in differentiation and mitochondrial function, only *aP2* was significantly lower in GPR120$^{-/-}$ mice (**Fig. 2F**). Interestingly, also many of the genes involved in substrate uptake were lower in GPR120$^{-/-}$ mice, including *Lpl*, *Angptl4*, *Glut1* and *Glut4* (**Fig. 2G**).

GPR120 deficiency does not affect plasma lipid metabolism

Since GPR120$^{-/-}$ mice displayed higher fat mass and lower markers of BAT activity, we studied whether the unfavorable metabolic phenotype of GPR120$^{-/-}$ mice coincided with deterioration of plasma lipid metabolism. After 8 weeks of HFD, plasma triglyceride (**Fig. 3A**), free fatty acid (**Fig. 3B**) and glucose levels (**Fig. 3C**) did not significantly differ between GPR120$^{-/-}$ and control mice. Likewise, GPR120 deficiency did not affect excursions of plasma triglyceride (**Fig. 3D**) and free fatty acid (**Fig. 3E**) levels in response to an oral olive oil gavage, suggesting unaltered plasma clearance of chylomicrons. To evaluate the relative contribution of organs in the plasma clearance of triglyceride-derived fatty acids, we next

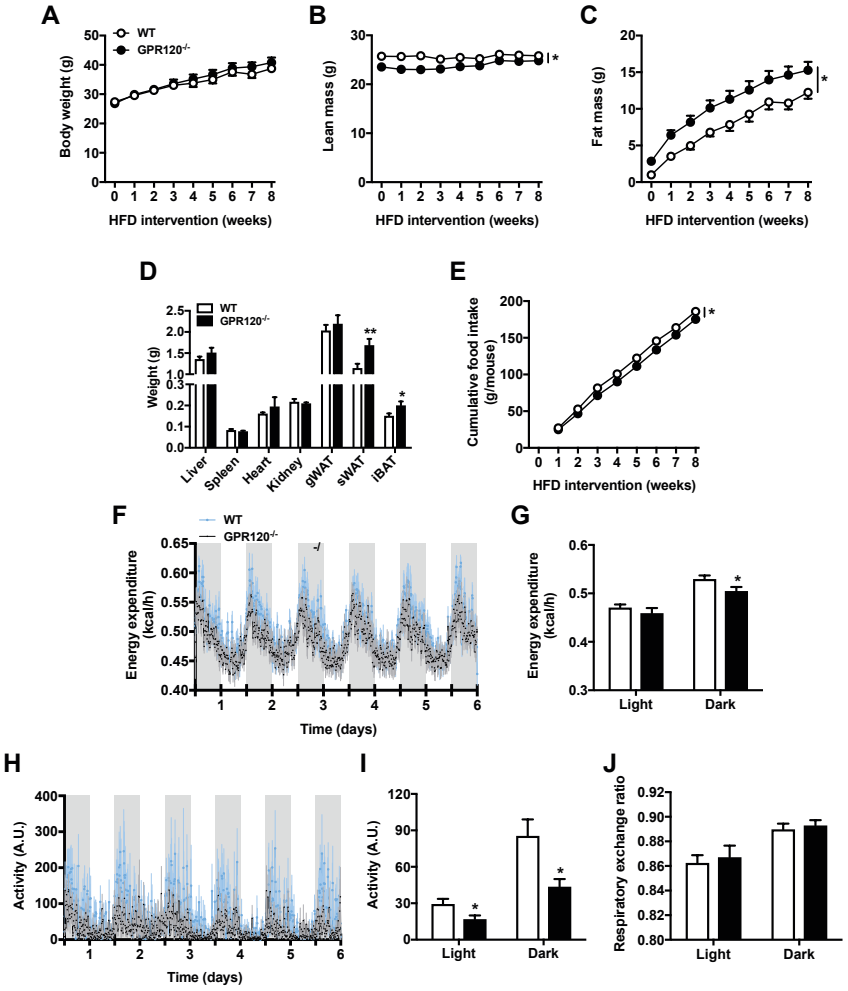

Figure 1. GPR120 deficiency increases HFD-induced fat accumulation and reduces physical activity. *GPR120$^{-/-}$ and wild-type (WT) mice were fed a high-fat diet (HFD) for 8 weeks. Body weight (A), lean mass (B), fat mass (C) and food intake (E) were measured weekly. At the end of the study, mice were sacrificed and organs were collected and weighed (D). In the second week of HFD, mice were housed in fully automatic metabolic cages (LabMaster System; TSE Systems), which measured oxygen uptake (V_{O2}) and carbon dioxide production (V_{CO2}). Total energy expenditure was calculated from V_{O2} and V_{CO2} using the Weir equation (F) and the average energy expenditure over the entire week was calculated for the light and dark phase separately (G). Physical activity was measured with infrared sensor frames (H) and the average physical activity over the entire week was calculated for the light and dark phase separately (I). Respiratory exchange ratio was calculated as the V_{CO2} / V_{O2} ratio (J). Values are presented as mean ± SEM (n=8-9). *p<0.05, **p<0.01 vs. WT.*

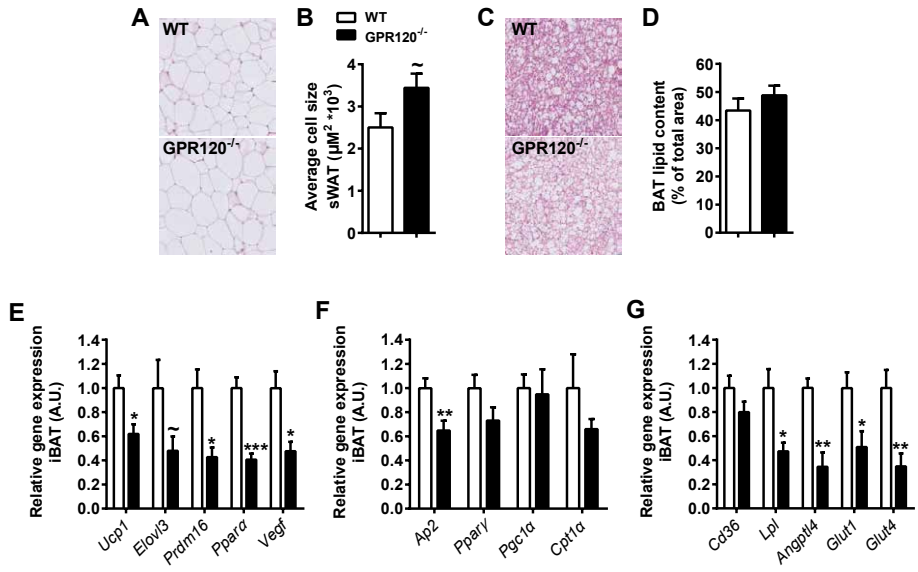

Figure 2. GPR120 deficiency reduces BAT-specific gene expression. *GPR120$^{-/-}$ and wild-type (WT) mice were fed a high-fat diet (HFD) for 8 weeks. Haematoxylin and Eosin staining of sWAT and iBAT sections was performed, and representative figures are shown (A, C). The cell size of adipocytes in sWAT (B) and the lipid content of iBAT (D) were quantified and representative pictures are shown. mRNA expression of the indicated genes was determined in iBAT (E-G). Values are presented as mean ± SEM (n=7-9). *p<0.05, **p< 0.01, ***p< 0.001 vs. WT.*

assessed the plasma clearance and organ distribution of [^{3}H]TO-labeled triglyceride-rich lipoprotein-like emulsion particles (**Fig. 3F**). While the clearance of [^{3}H]TO from plasma was unaffected, the uptake of [^{3}H]TO-derived oleate was higher in sWAT of GPR120$^{-/-}$ mice (**Fig. 3G**). This was due to the larger amount of sWAT in GPR120$^{-/-}$ mice, since uptake per gram sWAT was similar in both groups (data not shown). While the plasma clearance of [^{14}C] deoxyglucose (DG) also did not differ between GPR120$^{-/-}$ mice and controls (**Fig. 3H**), the uptake of [^{14}C]DG by the kidneys and iBAT of GPR120$^{-/-}$ mice was higher (**Fig. 3I**). Although this could suggest that deficiency of the lipid sensor GPR120 directs BAT to take up glucose, GPR120 deficiency does not have major impact on lipid metabolism.

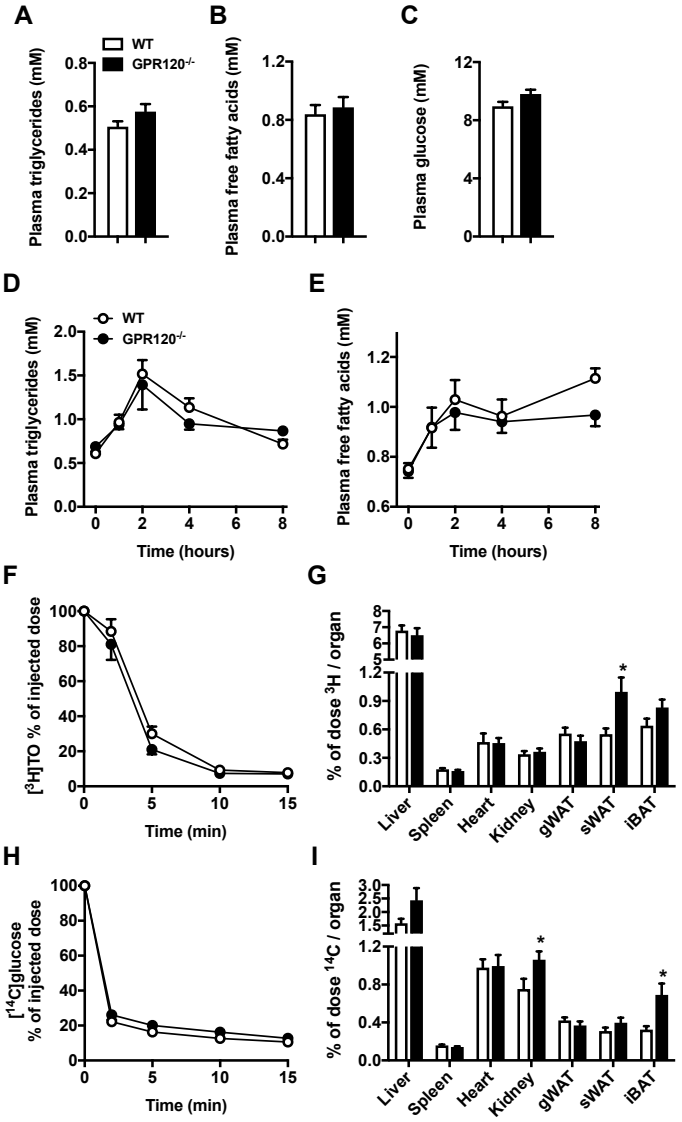

Figure 3. GPR120 deficiency does not affect plasma lipid metabolism. *GPR120$^{-/-}$ and wild-type (WT) mice were fed a high-fat diet (HFD) for 8 weeks. At the end of the study, 6 h fasted blood samples were drawn and plasma triglycerides (A), free fatty acids (B) and glucose (C) were determined. After 6 weeks of HFD, a basal blood sample was drawn from 4 h-fasted mice upon which they received an oral bolus of olive oil. Subsequently, at 1, 2, 4 and 8 hours after the olive oil gavage, blood samples were taken and assayed for plasma triglycerides (D) and free fatty acids (E). After 8 weeks of HFD, mice were injected i.v. with glycerol tri[^{3}H]oleate-labeled lipoprotein-like particles and [^{14}C]deoxy-D-glucose, and clearance from plasma (F, H) and uptake by organs at 15 min after injection (G, I) were determined by analyzing ^{3}H- and ^{14}C-activity. Values are presented as mean ± SEM (n=8-9). *p<0.05 vs. WT.*

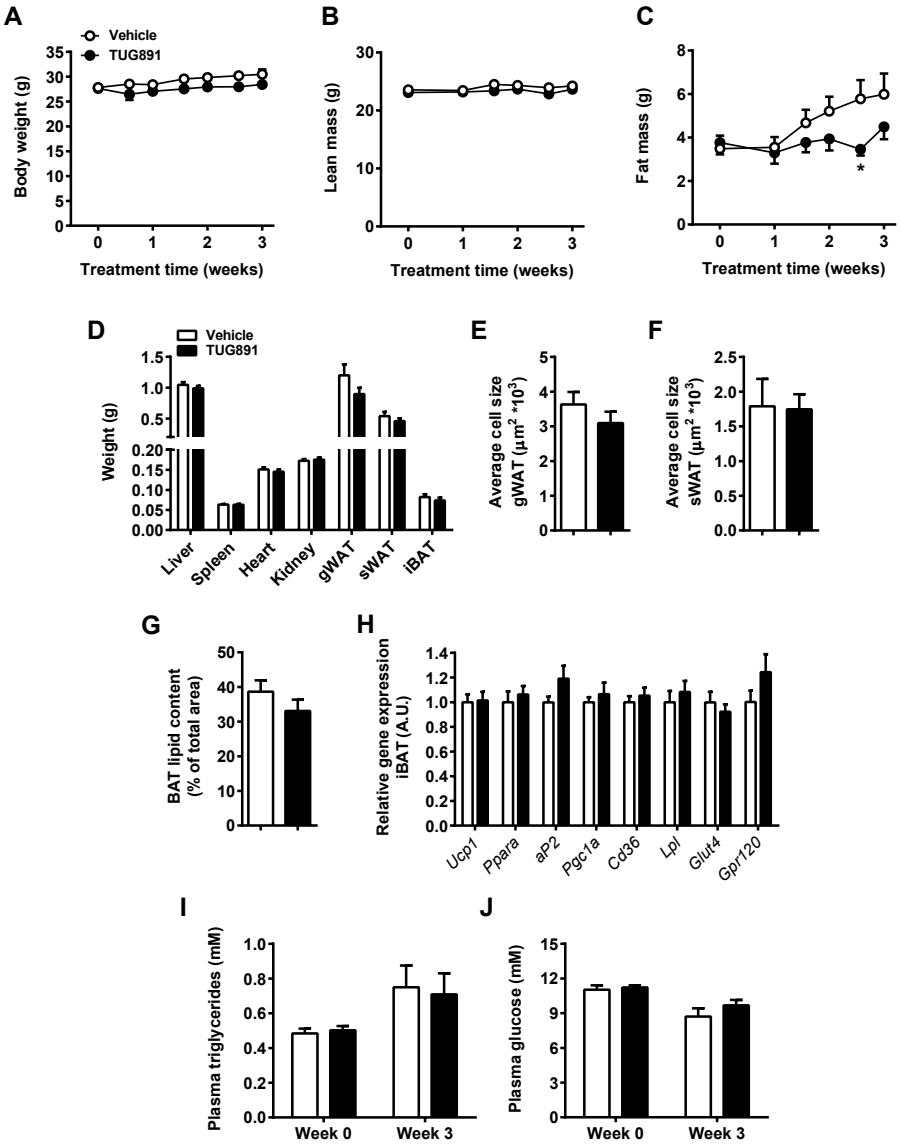

Figure 4. Oral treatment with GPR120 agonist TUG891 reduces fat mass. *Wild-type mice were fed a high-fat diet (HFD) for 6 weeks and treated orally with vehicle or 10 mg/kg body weight TUG891 during the last 3 weeks of HFD feeding. Body weight (A), lean mass (B) and fat mass (C) were measured once to twice weekly. At the end of the study, mice were sacrificed and organs were collected and weighed (D). Haematoxylin and Eosin staining of gWAT, sWAT and iBAT sections was performed, and the cell size of adipocytes in gWAT (E) and sWAT (F), and the lipid content of iBAT (G) were quantified. mRNA expression of the indicated genes was determined in iBAT (H). Before the start of TUG891 treatment and after 3 weeks, 6 h fasted blood samples were drawn and plasma triglycerides (I) and glucose (J) were determined. Values are presented as mean ± SEM (n=7-8). *p<0.05 vs. vehicle.*

Oral treatment with GPR120 agonist TUG891 reduces fat mass

To assess the therapeutic potential of targeting GPR120 to reduce fat mass, we next treated HFD-fed mice orally with the GPR120 agonist TUG891 (10 mg/kg daily) or vehicle for 3 weeks. Total body weight (**Fig. 4A**) and lean mass (**Fig. 4B**) were not affected by TUG891 treatment. However, fat mass was slightly reduced after 2.5 weeks of treatment (**Fig. 4C**). This was not due to a decrease in a specific adipose tissue depot, as gWAT, sWAT and iBAT weights were similar between TUG891-treated animals and vehicle-treated controls (**Fig. 4D**). Accordingly, TUG891 did not affect adipocyte cell size in gWAT (**Fig. 4E**) or sWAT (**Fig. 4F**), nor did it alter lipid droplet content (**Fig. 4G**) or gene expression (**Fig. 4H**) in iBAT. Plasma triglyceride (**Fig. 4E**) and glucose (**Fig. 4F**) levels were also unaffected by TUG891.

GPR120 agonist TUG891 acutely increases fat oxidation

Since GPR120 deficiency increased fat mass and reduced energy expenditure, and treatment with the GPR120 agonist TUG891 reduced fat mass in HFD-fed mice, we wanted to gain more insight into the mechanism behind the effect of GPR120 on energy utilization and fat mass. We first assessed the acute effects of TUG891 on energy expenditure and substrate utilization. Since the oral availability of compounds is often hindered by low and/or slow intestinal absorption but no data on optimal dosing was available yet, we chose to treat mice on a normal chow diet i.p. with increasing doses of TUG891 (twice daily). Strikingly, TUG891 injection before onset of the dark phase acutely decreased RER (**Fig. 5A**), increased fat oxidation (**Fig. 5B**) and decreased glucose oxidation (**Fig. 5C**), whereas TUG891 treatment at the beginning of the light phase did not have pronounced effects on these parameters. No differences were observed in total energy expenditure (**Fig. 5D**) or physical activity (**Fig. 5E**). Based on these initial results, we selected the intermediate dose of 35 mg/kg TUG891 and administration time 2 h before the initiation of the dark phase because of the prominent effects on fat oxidation. We then investigated in a larger number of mice (n=8) on a chow diet the effects of this dose of TUG891 i.p. once daily for a period of 5 consecutive days on RER and fat mass. While TUG891 did not affect total energy expenditure (**Fig. 6A, B**) or physical activity (**Fig. 6C, D**), the GPR120 agonist reduced RER (**Fig. 6E, F**), increased fat oxidation (**Fig. 6G, H**) and decreased glucose oxidation (**Fig. 6I, J**) during the dark phase. Total body weight (**Fig. 7A**) and lean mass (**Fig. 7B**) were unaffected. However, in accordance with the increased fat oxidation, fat mass tended to be reduced already after 5 days of treatment with TUG891 (**Fig. 7C**).

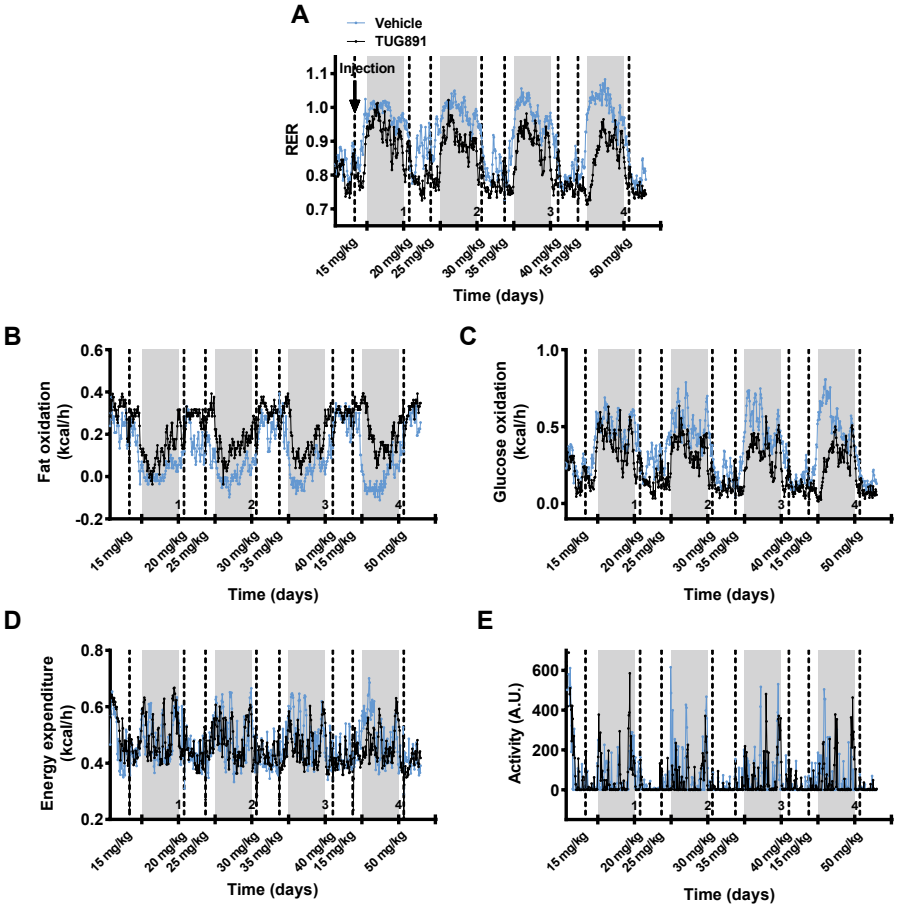

Figure 5. I.p. treatment with 35 mg/kg GPR120 agonist TUG891 acutely increases fat oxidation when injected before onset of the dark phase. *Wild-type mice were fed a chow diet and housed in fully automatic metabolic cages (LabMaster System; TSE Systems), which measured oxygen uptake (V_{O2}) and carbon dioxide production (V_{CO2}). Mice were i.p. injected (dotted line) twice daily at 9:00 and 16:00 with vehicle or increasing doses of TUG891 ranging from 15-50 mg/kg body weight as indicated over a period of 5 days. Respiratory exchange ratio (A), fat oxidation (B) and glucose oxidation (C) were calculated from V_{O2} and V_{CO2}. Total energy expenditure was also calculated from V_{O2} and V_{CO2} using the Weir equation (D). Physical activity was measured with infrared sensor frames (E). (n=1).*

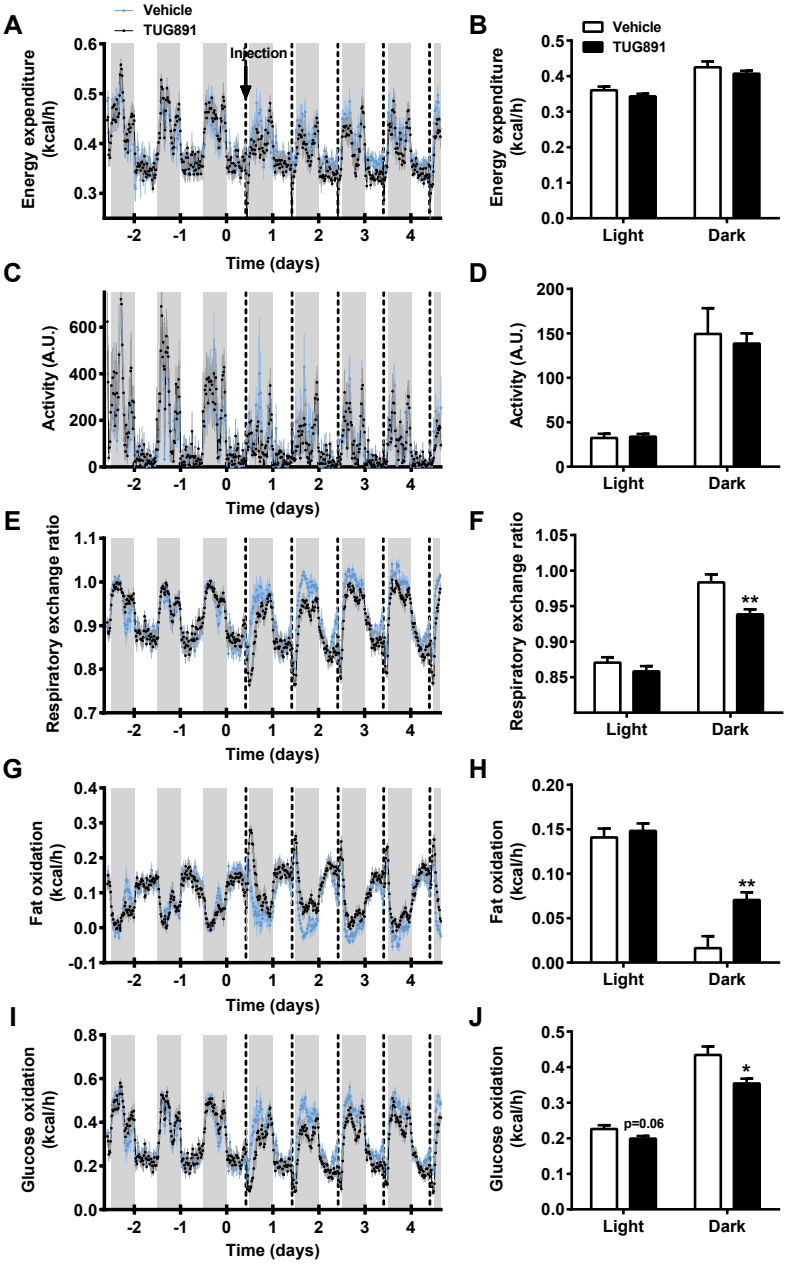
A
Vehicle
TUG891
Injection
Energy expenditure (kcal/h)
Time (days)
B
Vehicle
TUG891
Energy expenditure (kcal/h)
Light
Dark
C
Activity (A.U.)
Time (days)
D
Activity (A.U.)
Light
Dark
E
Respiratory exchange ratio
Time (days)
F
Respiratory exchange ratio
**
Light
Dark
G
Fat oxidation (kcal/h)
Time (days)
H
Fat oxidation (kcal/h)
**
Light
Dark
I
Glucose oxidation (kcal/h)
Time (days)
J
Glucose oxidation (kcal/h)
p=0.06
*
Light
Dark

Figure 6 (left page). GPR120 agonist TUG891 acutely increases fat oxidation. *Wild-type mice were fed a chow diet and housed in fully automatic metabolic cages (LabMaster System; TSE Systems), which measured oxygen uptake (V_{O2}) and carbon dioxide production (V_{CO2}). Mice were i.p. injected once daily (dotted line) at 17:00 with 35 mg/kg body weight TUG891 for 5 consecutive days. Total energy expenditure was calculated from V_{O2} and V_{CO2} using the Weir equation (A) and the average energy expenditure over the treatment period was calculated for the light and dark phase separately (B). Physical activity was measured with infrared sensor frames (C) and the average physical activity over the treatment period was calculated for the light and dark phase separately (D). Respiratory exchange ratio was calculated as the V_{CO2} / V_{O2} ratio (E) and the average respiratory exchange ratio over the treatment period was calculated for the light and dark phase separately (F). Fat oxidation (G) and glucose oxidation (I) were also calculated from V_{O2} and V_{CO2} and the average fat and glucose oxidation over the treatment period was calculated for the light and dark phase separately (H, J). Values are presented as mean ± SEM (n=8). *p<0.05, **p<0.01 vs. vehicle.*

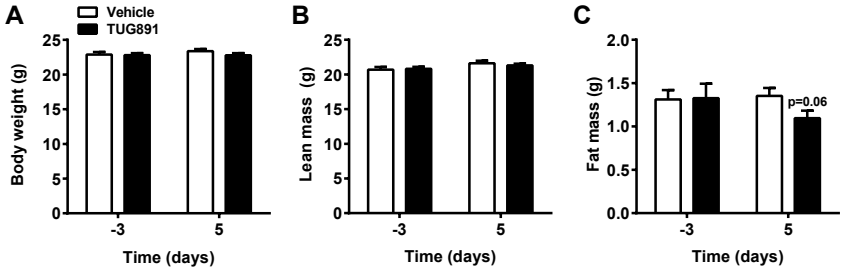

Figure 7. GPR120 agonist TUG891 tends to reduce fat mass. *Wild-type mice were fed a chow diet and i.p. injected once daily at 17:00 with vehicle or 35 mg/kg body weight TUG891 for 5 consecutive days. Body weight (A), lean mass (B) and fat mass (C) and were measured before and after TUG891 treatment. Values are represented as mean ± SEM (n=8).*

DISCUSSION

In the current study, we assessed the role of GPR120 in obesity development and lipid metabolism. We demonstrated that GPR120 deficiency, although not evidently influencing plasma lipid metabolism, increases HFD-induced fat mass and reduces energy expenditure. This coincided with lower physical activity and lower BAT-specific gene expression. In addition, GPR120 agonism reduced fat mass related to increased fat oxidation, which highlights the therapeutic potential of targeting GPR120 to reduce obesity.

We established that GPR120$^{-/-}$ mice exhibited higher fat mass as compared to wild-type control mice. This corroborates previous findings by Ichimura *et al.* (7), who reported that GPR120$^{-/-}$ mice on a HFD store more fat than wild-type controls. In our study, this increased fat mass of GPR120$^{-/-}$ mice was not reflected by higher body weight. This is in contrast to the previous study in which the authors did find that GPR120 deficiency increased HFD-

induced body weight gain (7). This discrepancy may be explained by differences in age or strain of the mice, or even the facility in which experiments were performed. We found that GPR120$^{-/-}$ mice had lower energy expenditure in the dark phase, which could contribute to their higher fat mass. Remarkably, lower energy expenditure in young GPR120$^{-/-}$ mice was also reported previously, but only during the light phase (7).

The most striking effect of GPR120 deficiency we observed was lower physical activity levels during both the light and the dark phase, which may partly underlie the lower energy expenditure of GPR120$^{-/-}$ mice. Although physical activity has not been investigated in relation to GPR120 before, reduced physical activity is often reported in mice that underwent orchidectomy (20, 21) or with androgen deficiency (21). Besides in adipose tissue, GPR120 is also highly expressed in the pituitary gland (6, 22). Moreover, GPR120 protein colocalizes with LHβ and FSHβ, being subunits of the gonadotropin hormones LH and FSH, respectively, in the pituitary gland (23). This suggests that GPR120 may stimulate gonadotropin secretion in the pituitary and thereby indirectly stimulate androgen production in the testes. In line with this hypothesis, fatty acids increase LH release from pituitary cells (22). However, it has also been described that the GPR40/120 agonist GW9508 does not induce LH release and the GPR40/120 antagonist GW1100 fails to inhibit fatty acid-induced LH release from pituitary cells, arguing against a role for GPR120 in the fatty acid-induced release of LH from the pituitary (22). Whether GPR120 regulates pituitary gonadotropin secretion *in vivo* and what the effect of GPR120 deficiency is on androgen levels is an interesting field of future investigation. As androgen deficiency exacerbates HFD-induced metabolic derangements in mice (21), low androgen levels could well be the mechanism behind the deteriorated metabolic phenotype (*i.e.* increased fat mass, less active BAT) we found in GPR120 deficient animals.

Another important contributor to energy expenditure is BAT. GPR120$^{-/-}$ mice in our study exhibited increased iBAT mass and reduced gene expression of BAT markers and genes involved in substrate uptake by BAT, indicating that GPR120 deficiency indeed renders BAT less active. Surprisingly, GPR120 deficiency was not accompanied by higher plasma lipid levels, worsened olive oil tolerance or slower plasma lipid clearance. Others have found high and inducible mRNA expression of *Gpr120* in BAT upon cold, and impaired cold-induced browning in GPR120$^{-/-}$ mice, both of which do suggest an important role of GPR120 in BAT activation (24). Thus, besides the lower physical activity we found in GPR120$^{-/-}$ mice, lower BAT activity may in part underlie the higher fat accumulation in WAT. However, even though GPR120 agonism activates BAT, is involved in browning of WAT (24), and both BAT and browned WAT are important regulators of lipid metabolism (25), the presence of GPR120 on BAT does not seem to contribute to fatty acid uptake by BAT or to regulate plasma lipid levels in our study.

In line with the observation that deficiency for GPR120 increased fat mass, agonism of GPR120 using the agonist TUG891 decreased fat mass. We selected TUG891 because of its high selectivity for GPR120 over GPR40 compared to other agonists such as GW9508 and NCG21 (15, 26). An oral dose of 10 mg/kg daily reduced fat mass after 2.5 weeks of treatment, whereas an i.p. dose of 35 mg/kg daily already tended to reduce fat mass after

5 days of treatment. Obviously, the higher dose in the latter experiment is probably mainly responsible for this difference, although the fact that the orally treated mice were on a HFD and the dissimilar bioavailability of the compound upon oral *vs.* i.p. treatment (lower absorption of compounds upon oral administration decreases their bioavailability) likely also contributed to the varying effects on fat mass. Only one study describing treatment of rodents with TUG891 has been published to date. In this study, mice received a daily dose of 20 mg/kg TUG891 through the drinking water for 2 weeks, upon which reduced fat mass, improved glucose and insulin tolerance were observed (27). A disadvantage of this administration route is that GPR120, which is also expressed on the tongue, may be involved in taste preference (28, 29). When treating mice i.p. twice daily, we observed larger effects of TUG891 on substrate utilization when administered at the end of the light phase than when administered at the beginning of the light phase. Besides dose and administration route, it is becoming increasingly common to take into account the circadian timing of administration to maximize therapeutic efficacy (30). Since lipid metabolism is also under circadian regulation (31), we believe that timing of TUG891 administration is of great importance to optimize the intended effect and minimize possible (toxic) side effects associated with administration of many exogenous compounds.

When further investigating the mechanism behind the potential of TUG891 to decrease fat mass, increased fat oxidation directly after administration of TUG891 was observed. This increase in fat oxidation is likely due to increased BAT activity, as previous studies have demonstrated that BAT activation increases fatty acid uptake by BAT (25, 32), and stimulates lipid oxidation rather than glucose oxidation (25). Others have shown that the GPR40/120 agonist GW9508 activates BAT and increases oxygen consumption in mice. Whether TUG891 actually increases triglyceride-derived fatty acid uptake by BAT and whether the stimulation of fat oxidation is mediated by GPR120 should be assessed in future studies.

In humans, GPR120 is well expressed in both subcutaneous and omental adipose tissue (7). Besides, branched fatty acid esters of hydroxy fatty acids (FAHFAs), a novel class of endogenous lipids, signal through GPR120 and positively correlate with insulin sensitivity in humans (33). These findings strengthen the possibility that the metabolic effects of GPR120 activation are translatable to humans.

In conclusion, absence of the fatty acid receptor GPR120 deteriorates metabolic phenotype, evidenced by increased fat mass and reduced physical activity in HFD-fed mice, without evidently altering plasma lipid metabolism. The GPR120 agonist TUG891 increases fat oxidation and reduces fat mass, demonstrating that GPR120 agonism, and this specific compound, hold therapeutic potential to reduce obesity. Further research into the mechanisms behind the beneficial metabolic effects of TUG891 is warranted to further develop this compound for treatment of obesity.

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