Article

Profiling of G-protein Coupled Receptors in Adipose Tissue and Differentiating Adipocytes Offers a Translational Resource for Obesity/Metabolic Research

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Abstract: G protein-coupled receptors (GPCRs) are expressed essentially on all cells, facilitating cellular responses to external stimuli, and are involved in nearly every biological process. Several members of this family play significant roles in the regulation of adipogenesis and adipose metabolism. However, the expression and functional significance of a vast number of GPCRs in adipose tissue are unknown. We used a high-throughput RT-PCR panel to determine the expression of the entire repertoire of non-sensory GPCRs in mouse white, and brown adipose tissue and assess changes in their expression during adipogenic differentiation of murine adipocyte cell line, 3T3-L1. In addition, the expression of GPCRs in subcutaneous adipose tissues from lean, obese, and diabetic human subjects was evaluated by re-analyzing RNA-sequencing data. We detected a total of 292 and 271 GPCRs in mouse white and brown adipose tissue, respectively. There is a significant overlap in the expression of GPCRs between the two adipose tissue depots but several GPCRs are specifically expressed in one of the two tissue types. Adipogenic differentiation of 3T3-L1 cells had a profound impact on the expression of several GPCRs. RNA sequencing of subcutaneous adipose from healthy human subjects detected 255 GPCRs and obesity significantly changed the expression of several GPCRs in adipose tissue. Finally, we report several highly expressed GPCRs with no known role in adipose biology whose expression was significantly altered during adipogenic differentiation and/or in the diseased human subjects. These GPCRs could play an important role in adipose metabolism and serve as a valuable translational resource for obesity and metabolic research.

Keywords: adipose tissue; adipogenesis; G-protein-coupled receptors; thermogenesis; obesity; metabolic syndrome

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1. Introduction

The G-protein coupled receptors (GPCRs) are the largest family of membrane proteins and are expressed widely in the body. They regulate several important biological processes, and dysregulation of GPCR signaling has been implicated in the pathogenesis of many diseases (1-3). There are ~800 members of the GPCR family and more than 400 are sensory receptors (olfactory, vision, and taste receptors). The remaining ~356 are non-sensory receptors and are activated by physical ligands (4). The unique blend of variety and specificity within the GPCR family and the fact that they are readily targetable by exogenous drugs make GPCRs attractive therapeutic targets (5-6). 30-40% of the currently marketed drugs target GPCRs. However, the overall number of targeted GPCRs is about 30% and a vast number of GPCRs are yet to be exploited for therapeutic purposes. In adipose tissue, GPCRs regulate critical processes such as adipogenesis, lipolysis, thermogenesis, glucose metabolism, and secretion of adipokines (7-10). Of all the GPCRs, β-adrenergic receptors (βARs) have been comprehensively studied for their role in adipose metabolism (11-14). All three members of the βAR family (β1, β2, and β3) are expressed in adipose with β3 AR being the predominant one. βARs are stimulated by norepinephrine and signaling occurs via the activation of G-protein subunit Gαs leading to the accumulation of second messenger cAMP that phosphorylates and activates its downstream targets including cAMP-dependent protein kinase A (PKA). Activation of PKA leads to increased lipolysis via hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), and perilipin (11). βARs are also involved in thermogenesis in brown adipose tissue (15). In addition to the βARs, several other GPCRs have been shown to play important functional roles in white and brown fat including alpha-adrenergic receptors (16), free fatty acid receptors (7, 17, 18), adenosine receptors (19), adhesion receptors (20-21), hydroxycarboxylic acid receptors (22,23), and many others (24). These still represent a significantly small fraction of the total number of GPCRs and the functional significance of a vast majority of GPCRs is not known in adipose metabolism. This is to a certain extent due to the inadequate data regarding their expression in adipose tissue and differentiating adipocytes. Therefore, this study was initiated to comprehensively profile the entire repertoire of non-visual GPCRs using high-throughput RT-PCR in mouse brown and white adipose tissue and during adipogenic differentiation of 3T3-L1 cells. Besides, the expression of GPCRs in subcutaneous adipose tissues from healthy lean, healthy obese and unhealthy obese human subjects was assessed by re-analyzing RNA-sequencing data # GSE152991 (25). Our comprehensive analysis revealed several GPCRs with no known role in adipose biology that were highly expressed in adipose tissue and demonstrated significant changes in expression during adipogenesis and/or in diseased human subjects. Understanding the role of these recently discovered GPCRs in the metabolism of adipose tissue could lead to the discovery of previously unrecognized disease-drug relationships and speed up the process of developing new drugs for obesity and metabolic disorders.

2. Materials and Methods

2.1. Reagents and Chemicals

Mouse 3T3-L1 cell lines were purchased from American Type Culture Collection (ATCC), (Manassas, VA, USA). The cell culture reagents including Advanced Dulbecco's modified Eagle's medium (DMEM), GlutaMAX, Penicillin-Streptomycin (10,000 U/mL), and fetal bovine serum (FBS) were ordered from Gibco Life Technologies (Thermo Fisher Scientific, USA). The Oil Red O solution, 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, Rosiglitazone, and insulin were obtained from Sigma-Aldrich (St. Louis, MO, USA). PureLink RNA purification kit by Ambion, cDNA synthesis kit, TaqMan Array Mouse GPCR Panel, advanced TaqMan PCR Master Mix, and SYBR Green PCR Master Mix were purchased from Applied Biosystem (Thermo Fisher Scientific, USA). DNA oligonucleotides were obtained from Macrogen. Antibodies were obtained from Thermo Fisher (Thermo Fisher Scientific, USA) and Cell Signaling Technology (Danvers, MA, USA).

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2.2. Culture and adipogenic differentiation of 3T3-L1 adipocytes

Cells were maintained in the complete advanced DMEM media until confluence. Two to three days post-confluence, the differentiation was induced using an adipogenic cocktail which included 0.25 µM dexamethasone, 0.5 mM IBMX, 3 µM Rosiglitazone, and 1 µM insulin in advanced DMEM with 10% FBS. Four days post differentiation the culture medium was changed with advanced DMEM + 10% FBS+ 1µM Insulin for 24 h (post-differentiation). Cells were maintained in advanced DMEM with 10% FBS and used on Day 7 of differentiation.

2.3. Isolation of mouse adipose tissue depots

All protocols and procedures were approved by the Institutional Research Ethics Committee (REC) at King Saud University in Riyadh, Saudi Arabia (approval no. KSU-SE-18-39). C57BL/6 male mice from Taconic Biosciences, USA, were housed in a room temperature set at 22-24 °C and 45% humidity under 12 h light/dark cycles daily. At 10 weeks of age, 5 mice were sacrificed and both inguinal subcutaneous white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were collected, snap-frozen in liquid nitrogen, and stored at − 80 °C until analysis.

2.4. RNA isolation and RT-PCR by Taqman arrays

Total RNA was extracted from mouse adipose tissue depots using Trizol reagent and isolated using the chloroform-isopropanol method. To remove genomic DNA contamination, RNA was purified using DNase (5 PRIME). RNA was isolated from cultured 3T3L1 cells using an RNeasy kit with DNase treatment (Qiagen, Valencia, CA, USA). Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Gene expression array was performed on cDNA samples from 3T3-L1 cells and mouse adipose tissue depots and GPCR expression was determined with TaqMan Array Mouse GPCR Panel (Applied Biosystems, Life Technologies Corp., Carlsbad, CA, USA) according to the recommended protocol, run on an ABI Prism 7900HT system (Applied Biosystems), and analyzed with the Sequence Detection System software (Applied Biosystems). These arrays are efficient, easy-to-use microfluidic cards for quantitative gene expression analysis of GPCR genes. The expression of 352 non-visual GPCRs was determined with these arrays. GPCR expression was quantified relative to the 15 housekeeping genes (Actb, B2m, Gapdh, Gusb, Hmbs, Hprt1, Ipo8, Pgk1, Polr2a, Ppia, Rplp2, Tbp, Tfrc, Ubc, Ywhaz) in the sample. Based on the relative expression, GPCR mRNA levels were sub-divided as “Expressed”, “Trace” and absent/non-quantifiable as described by Patricio Atanes et al (26). Genes in the expressed category were present > 0.1% of the mRNA expression of 15 housekeeping genes, while GPCRs with a relative expression between 0.01% to 0.1% were present at trace levels. Those GPCRs that were either not detected or present at <0.01% of endogenous controls were considered “Absent”. This roughly translates to Ct values of <30, 30-33, and >33 for “Expressed”, “Trace” and “Absent” GPCRs, respectively.

2.5. Western blotting

Cell pellets were lysed in RIPA buffer (50 mM Tris; pH 7.5, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 2 mM EDTA, and 1% Triton X-100) containing protease inhibitor (name). The lysates were incubated on ice for 15 minutes followed by the centrifugation for 15 minutes at 15,000 g at 4 C. The clear lysate was transferred to a new 1.5 ml Eppendorf tube and protein quantification was done using Qubit Protein Assay Kit (Thermo fisher Scientific, USA). Separation of proteins by SDS-PAGE, immunoblotting was done with the indicated primary antibodies, and secondary HRP-conjugated antibodies using enhanced chemiluminescence detection reagents.

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2.6. RNA sequencing re-analysis

RNA sequencing dataset GSE152991 analyzed by the GEO RNA-seq Experiments Interactive Navigator (GREIN) platform (25). This dataset contained RNA sequencing data of subcutaneous adipose tissue from 45 human subjects. This includes metabolically healthy lean (MHL) (n = 11) had BMI 18.5–24.9 kg/m2, plasma TG concentration <150 mg/dL, fasting plasma glucose concentration <100 mg/dL, 2-hour OGTT plasma glucose concentration <140 mg/dL, HbA1c ≤5.6%, and IHTG content <4%. Metabolically healthy obese (MHO) (n = 15) had BMI 30–49.9 kg/m2, plasma TG concentration <150 mg/dL, fasting plasma glucose concentration <100 mg/dL, 2-hour OGTT plasma glucose concentration <140 mg/dL, HbA1c ≤5.6%, and IHTG content <4%. Metabolically unhealthy obese (MUO) (n = 20) had BMI 30–49.9 kg/m2, prediabetes (fasting plasma glucose concentration ≥100 mg/dL, 2-hour OGTT plasma glucose concentration ≥140 mg/dL, and/or HbA1c ≥5.7%), and IHTG content ≥5%. CPM-normalized mRNA expression was obtained form GREIN) platform and differential gene expression was estimated. Raw P values were adjusted for multiple testing using the Benjamini–Hochberg procedure and only adjusted P (Padj) values higher than 0.05 were considered significant. The expression of 356 non-visual GPCRs was determined in these samples.

2.7. Statistical analyses

Data are presented as the mean ± standard error of mean (SEM) values. Two-tailed Student's t-test was used to determine significant differences. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3. Results

3.1. GPCR expression profile in mouse white and brown adipose tissue

Taqman qPCR arrays were able to detect of 292 GPCRs in mouse white adipose tissue. 267 of these were expressed above trace levels, 25 at trace levels, and 60 were not detected by the arrays (Fig. 1A). On the other hand, brown adipose tissue expressed 271 GPCRs of which 196 were detected above trace levels, 75 were detected at trace levels and 81 were absent or undetected (Fig. 1B). The majority of GPCRs were expressed in both WAT and BAT but a significant number were differentially expressed in only one of the two adipose tissue types (Fig. 1C). Lysophosphatidic acid receptor 6 (Lpar6) also known as P2ry5 was the highest expressed receptor in both WAT and BAT followed by frizzled Class Receptor 4 (Fzd4). Adhesion receptor 5 (Adgrf5), and orphan receptor Gprc5b were also highly expressed in both adipose tissue types (Fig. 1D). GPCRs that are expressed in both adipose tissue depots but enriched in WAT include chemokine receptors (Cxcr5 and Ccr6), mas-related G-protein coupled receptor member D (Mrgprd), relaxin family peptide receptor 3 (Rxfp3) and 5-hydroxytryptamine (serotonin) receptor 1F (Htr1f) (Fig. 1E). Those enriched in BAT include leucine-rich repeat-containing G-protein coupled receptor 6 (Lgr6), galanin receptor 1 (Galr1), secretin receptor (SCTR), free fatty acid receptor 4 (Ffar4), sphingosine-1-phosphate receptor 5 (S1pr5) and P2Y purinoreceptor 1 (P2ry1) (Fig. 1F). A number of GPCRs are exclusively expressed in one of two adipose depots and absent/undetected in the other. The complete list of GPCRs that are only expressed in only one of the two adipose tissue depots is shown in table 1.

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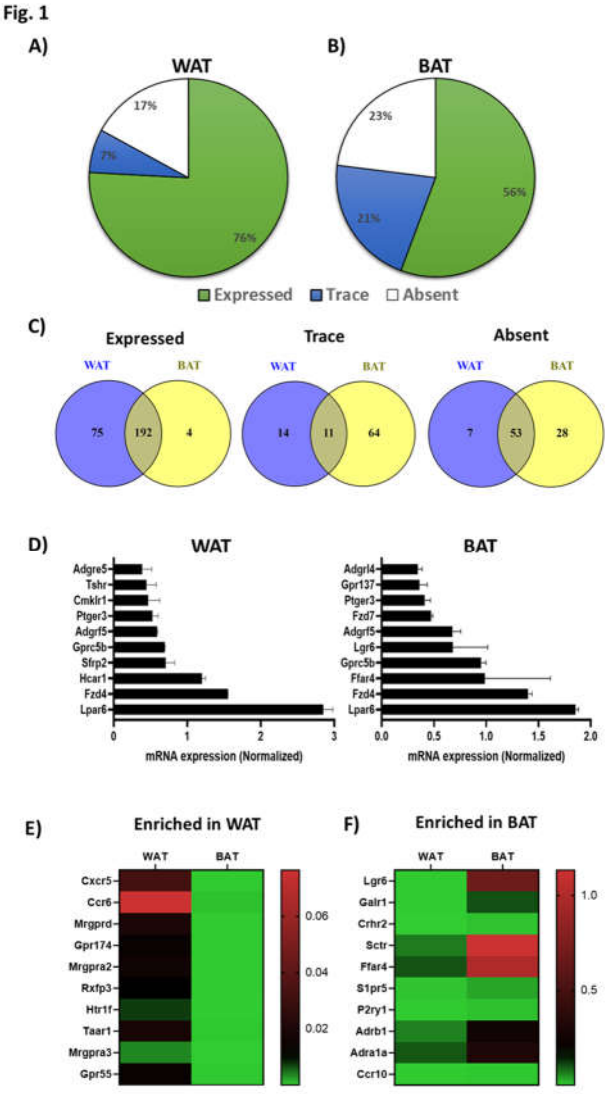

Figure 1 GPCRs expression in mouse White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT). A) Pi chart showing the proportion of expressed, trace and absent/undetected GPCRs in mouse WAT and BAT. B)Venn diagrams showing the relationship (distinct or overlapping) between the expressed, traced and absent/undetected GPCRs identified in WAT and BAT mouse tissues. C) Comparison of mouse mRNA expression of the top 10 expressed GPCRs between WAT and BAT. D) Heat maps showing normalized gene expression profiles for ten most enriched GPCRs in WAT and Bat. Data were generated using pooled RNA of WAT and BAT samples obtained from five mice. Each point represents an average mRNA expression of two independent experiments. The data are normalized to the average of 15 reference genes in the same samples as described in the method section.

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Table 1. List of GPCRs that are only detected in one of the two mouse adipose tissue depots (WAT or BAT).

Only in WAT	Only in BAT
Mrgprb3	Lpar1
Mrgpra1	Tacr2
Ffar3	Adgrf3
Mrgpra3	Nmbr
Gpr12	Adgrb3
Ltb4r2	Mchr1
Gpr119	Drd2
Npy2r	
Gpr6	
Tacr1	
Npy5r	
Npbwr1	
Oxgr1	
Rgr	
Grm4	
Gpr83	
Hrh4	
Hcrtr2	
Ptgdr	
Gpr139	
Glp2r	
Npsr1	
Cckar	
Tacr3	
Glp1r	
Galr3	
Grm6	
Nmur1	

3.2. Comparative analysis of GPCR mRNA expression in undifferentiated and differentiated 3T3-L1 adipocytes

3T3-L1 cells were differentiated into adipocytes using the mentioned cocktail. Oil red O, which stains neutral lipids and triglycerides was used to validate differentiated adipocytes. Oil red O stained multiple droplets in differentiated adipocytes compared to pre-adipocytes that did not show any lipid droplets (Fig. S1a). Additional validation of the differentiation process was done by evaluating the mRNA and protein expression of key adipogenic markers (Fig. S1b and S1c).

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Fig. S1

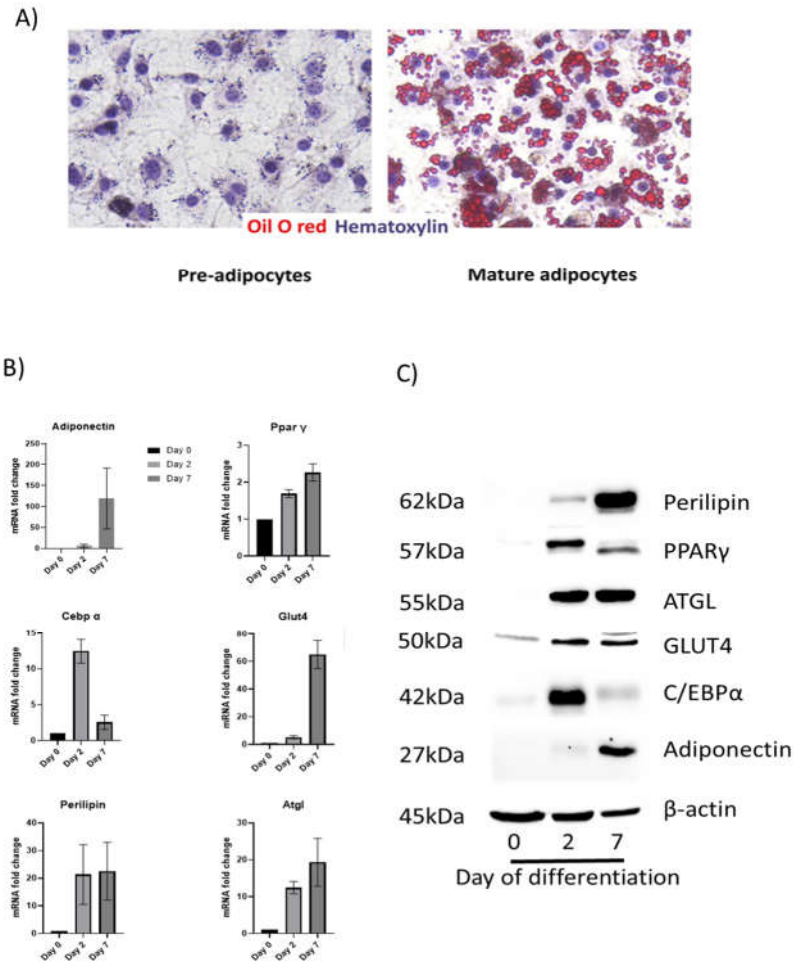

Figure S1. Adipogenic differentiation of 3T3 L1 cells and expression of key adipogenic markers. A)Oil-O red staining of 3T3 L1 cells before and after differentiation. B) mRNA expression and C) protein expression of C/EBP-α and PPAR-γ, Glut4, Hsl, Atgl, Adiponectin receptor and Adiponectin during the differentiation of 3T3 L1 adipocytes.

In undifferentiated 3T3-L1 pre-adipocytes, TaqMan Arrays detected 250 GPCRs of which 214 were detected above trace levels, 36 were expressed at trace levels and 102 were absent/undetected. On day 2 of differentiation 234 GPCRs were detected of which 123 were expressed at trace levels and 111 were expressed above trace levels. 118 GPCRs were undetected/absent. Fully differentiated 3T3-L1 adipocytes (day 7) expressed a total of 231 GPCRs of which 132 were expressed above trace levels, 99 at trace levels, and 121 were undetected or absent (Fig. 2A). The Venn diagrams in Figure 2B show these three groups with stratification of GPCR mRNA expression and the extent of commonality in 3T3-L1 cells at different stages of differentiation. The highly expressed GPCRs in 3T3-L1 cells at different stages of differentiation are shown in Figure 2C. Fzd1, Secreted Frizzled Related Protein 2 (Sfrp2), Calcitonin Receptor-like Receptor (Calcrl), Lpar1, and orphan receptor (Gpr153) are among the highly expressed GPCRs in undifferentiated 3T3-L1 cells (day 0). Gprc5b, Lgr4, SMO (smoothened), Fzd1, and Fzd4 are among the highly expressed GPCRs on Day 2. The list of GPCRs highly expressed in differentiated adipocytes (Day 7) includes Gprc5b, F2r (Coagulation Factor II Thrombin Receptor), Gpr137, Fzd4, and Lgr4 (Fig. 2C). Adipogenic differentiation of 3T3-L1 cells profoundly impacts the expression GPCRs as shown in the volcano plots (Fig. 2D). The expression of a large number of GPCRs is down-regulated whereas fewer GPCRs show an increased expression during the differentiation

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process. GPCRs that undergo significant upregulation during adipogenesis include Ffar4, Gprc5c (orphan GPCR), Gipr (Gastric inhibitory peptide), Hcar2, Gpr35, and Adrb2. Highly downregulated GPCRs include Secreted frizzled-related proteins (Sfrp1, Sfrp2, and Sfrp3), orphan GPCRs (Gpr176 and Gpr149), Neuropeptide Y Receptor Y1 (Npy1R), and Gpr153. Heat maps depicting the highly upregulated GPCRs (Fig. 2E) and highly downregulated GPCRs (Fig. 2F) during the differentiation process. GPCRs that are upregulated during the adipogenesis process and their functional significance in adipose are shown in the table 2.

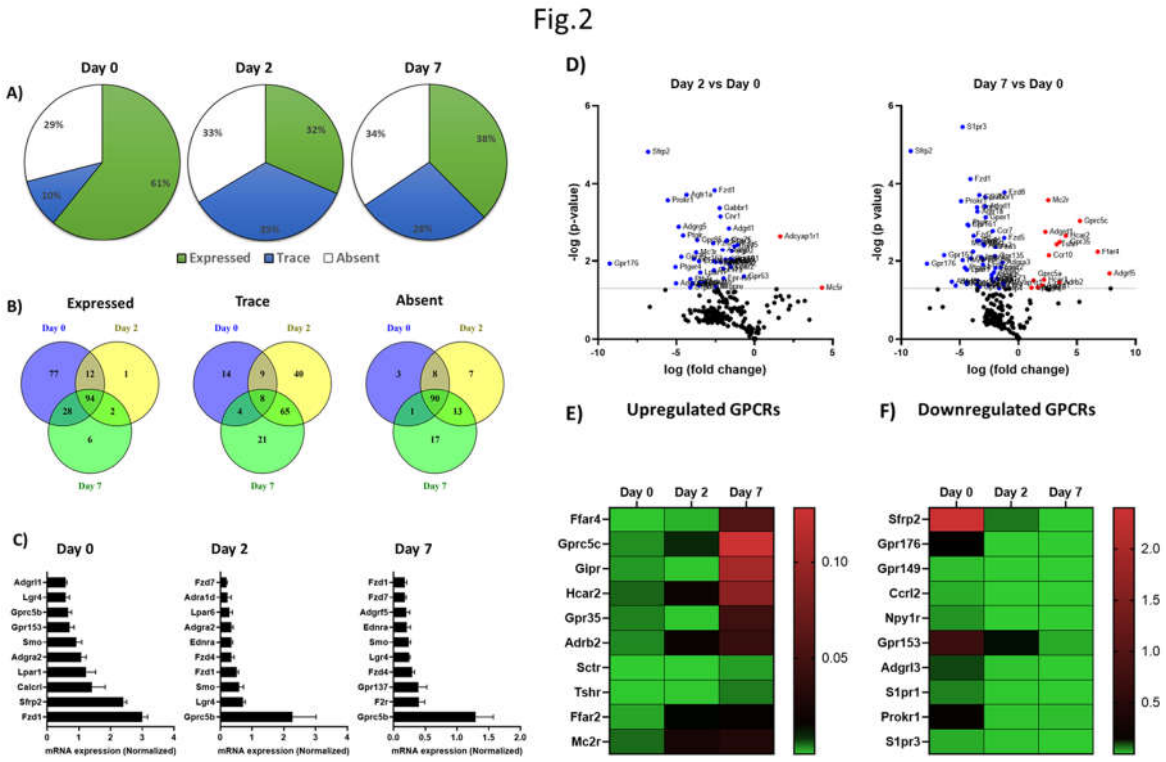

Figure 2. The GPCR mRNA expression during the adipogenic differentiation of 3T3 L1 cells. A) The fraction of the expressed, trace or absent GPCRs in the pre-adipocytes (Day 0), early adipocytes (Day 2) and mature adipocytes (Day 7). B) Venn diagrams showing the exclusive or shared genes for expressed, trace or absent mouse GPCR mRNAs from at Day 0 (Blue), at day 2 (Yellow) and at day 7 (Green). C) D) Volcano plot showing differentially expressed GPCRs genes, with -log (p-values) plotted against log (fold-changes) in Day 2 and Day 7 as compared to Day 0. GPCRs shown in color show a significant difference in expression with -log (p-values) more than 1.3 which corresponds to p-values<0.05. The GPCRs shown in red are upregulated whereas those in blue are downregulated. E) Heat maps showing normalized expression values for the 10 most downregulated (F) and upregulated (G) GPCRs during the differentiation of 3T3 L1 adipocytes. Each Data point represents an average of three independent experiments. The data are normalized to the average of 15 reference genes in the same sample as described in the method section.

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Table 2. List of GPCRs that are significantly upregulated during adipogenic differentiation of 3T3-L1 cells and their functional significance in adipose tissue biology.

Name	Log Fold change	Role in adipose
Adora1	7.865386	Induces lipolysis and increases energy expenditure
Adgrf5	7.774302	promotes insulin signaling and insulin-mediated glucose uptake
Ffar4	6.765778	Enhances insulin sensitivity, increases glucose uptake and thermogenesis.
Gprc5c	5.247853	Unknown
Gipr	5.197849	Enhances insulin sensitivity, increases glucose uptake and lipoprotein lipase activity.
Hcar2	4.04649	Unknown
Gpr35	3.526624	Regulates lipid metabolism, thermogenic, and anti-inflammatory gene expression in adipose tissue
Adrb2	3.493813	Regulates lipolysis and thermogenesis
Sctr	3.402992	Induces lipolysis
Tshr	3.266825	Regulates BAT adipogenesis
Ffar2	2.625755	
Ccr10	2.592673	Unknown
Mc2r	2.541011	Unknown
Mc5r	2.435111	promotes lipolysis and impairs re-esterification in adipocytes
Adgrd1	2.292871	Unknown
Hcar1	2.172346	Inhibits lipolysis and stimulates glucose uptake
Adgrg1	2.029279	Regulated adipogenesis
Adgrg7	1.64874	Unknown
Gprc5a	1.279647	Unknown
Gpr146	1.114973	Unknown
Kiss1r	1.020995	Promotes adipocyte differentiation and fat accumulation
Gprc5b	0.974769	Activates obesity-associated inflammatory signaling in adipocytes
Gpr17	0.948663	Unknown
F2r	0.738862	Unknown
Gpr137	0.717915	Unknown

3.3. GPCR expression in human subcutaneous adipose tissue and the impact of obesity

RNA sequencing detected a total of 257 GPCRs in subcutaneous adipose of healthy human subjects of which 172 were expressed above trace levels, 85 were expressed at trace levels whereas 99 GPCRs were absent or not detected (Fig. 3A). In terms of relative expression, the most abundant GPCRs include Frizzled Class Receptor 4 (FDZ4), Adhesion G Protein-Coupled Receptor (ADGRF5, ADGRL2, ADGRL4, ADGRA2), Prostaglandin E Receptor 3 (PTGER3), Calcitonin receptor-like (CALCRL), Neuropeptide Y receptor Y1 (NPY1R) and Leucine-Rich Repeat Containing G Protein-Coupled Receptor 4 (LGR4) (Fig. 3B). Obesity has a substantial effect on the expression of GPCRs and several members of this family show significant changes in their expression in both healthy and unhealthy obese human subjects (Fig. 3C-E). Heat maps showing the top 10 GPCRs that are down-regulated (Fig. 3F) or upregulated (Fig. 3G) in healthy obese and unhealthy obese subjects. The list of highly upregulated GPCRs includes Chemokine receptors (CCR5, C3AR1, CCR1), GPR183, Formyl peptide receptor (FPR3), GPR132, and adhesion GPCR (ADGRG4). Top downregulated GPCRs in obese subjects include Calcitonin receptor-like

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(CALCRL), Prokineticin receptor 1 (PROKR1), GPR146, LGR4, Adhesion G Protein-Coupled Receptors (ADGRL2 and ADGRB3), GIPR, Angiotensin II Receptor Type 1 (AGTR1), arginine vasopressin receptor 1A (AVPR1A) and ADRβ3.

Fig.3

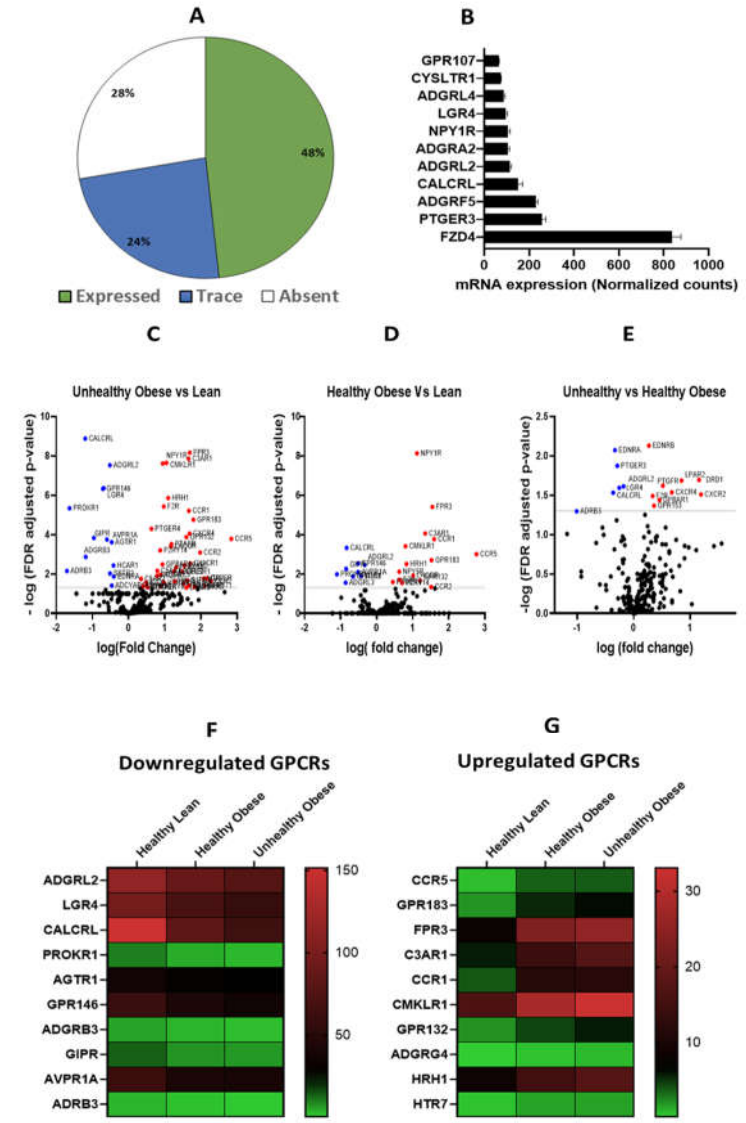

Figure 3. The GPCR mRNA repertoire in the subcutaneous adipose tissue of healthy lean, healthy obese and unhealthy obese human subjects from the RNA sequencing data set GSE 152991. A) The proportion of the expressed, trace and undetected GPCRs and B) the most abundant GPCRs in the subcutaneous adipose of healthy human subjects. C) Volcano plots showing differentially expressed GPCRs (FDR adjusted p-values <0.05) in color, with FDR plotted against fold-change in subcutaneous adipose of unhealthy obese subjects versus lean (C), healthy obese versus lean (D), and unhealthy (E) against healthy subjects. The GPCRs shown in red are upregulated whereas those in blue are downregulated Heat maps showing individual expression values for the 10 most downregulated (F) and upregulated (G) GPCRs in subcutaneous adipose tissue of human subjects without or with obesity. Each data point represent normalized mRNA counts from healthy lean (N=11), healthy obese (N=14), and unhealthy obese (N=20) as described in the method section.

3.4. GPCRs of interest

Our comprehensive analysis revealed several GPCRs that had remarkable expression profile, which includes 1) high expression in human adipose tissue 2) significant changes

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in the diseased state and/or 3) significant upregulation during the adipogenic differentiation of 3T3 L1 cells. GPCRs whose expression is significantly altered in adipose tissue of obese human subjects are listed in table 3A (downregulated) and table 3B (upregulated). Many GPCRs in this list have previously been studied for their role in adipose biology. These include alpha- and beta-adrenergic receptors (11-16), adenosine receptors (19), free fatty acid receptors (7, 17, 18), adhesion receptors (20, 21), hydroxycarboxylic acid receptors (22, 23), gastric inhibitory peptide (27-29), chemokine receptors (30-31) and many others (32-35). Interestingly, many GPCRs with notable expression profiles in adipose tissue and adipocytes have no known role in adipose metabolism. Here is the description of these important GPCRs that warrant further research to investigate their adipose-specific functional significance and therapeutic potential.

Table 3A. List of GPCRs that are downregulated in the subcutaneous adipose tissue of obese human subjects and their expression profile in mouse adipose tissue and 3T3 L1 adipocytes.

GPCR Name	Log Fold change (Human adipose)		Log Fold hange 3T3-L1	Expression in Mouse Adipose		Previously studied in adipose
	Healthy obese vs Lean	Unhealthy obese vs Lean	Day 7 vs Day 0	WAT	BAT	
GPR50	-0.680	-2.517	Not detected	No	No	Yes
GPR179	-0.657	-2.153	Not detected	No	No	No
ADRB3	-0.759	-1.694	2.36	Yes	Yes	Yes
PROKR1	-1.054	-1.666	-4.89	Yes	Yes	yes
SSTR1	-0.387	-1.322	-1.81	Yes	Yes	Yes
ADGRB3	-0.635	-1.237	Not detected	No	Yes	No
CALCRL	-0.835	-1.206	-3.56	Yes	Yes	Yes
GIPR	-0.838	-0.955	5.19	Yes	Yes	Yes
GPR146	-0.520	-0.725	1.11	Yes	Yes	No
CHRM3	-0.441	-0.709	-2.7	Yes	Yes	No
LGR4	-0.428	-0.695	-1.26	Yes	Yes	Yes
GPR63	-0.590	-0.653	-0.05	Yes	Yes	No
AVPR1A	-0.521	-0.608	ND	Yes	Yes	No
ADGRL2	-0.341	-0.528	-2.28	Yes	Yes	No
SSTR2	-0.314	-0.519	0.67	Yes	Yes	No
AGTR1	-0.309	-0.489	-3.50	Yes	Yes	NO
ADCYAP1R1	-0.441	-0.473	-1.30	Yes	Yes	Yes
HCAR1	-0.341	-0.426	2.17	Yes	Yes	Yes
EDNRA	-0.080	-0.418	-0.57	Yes	Yes	Yes

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Table 3B. List of GPCRs that are upregulated in the subcutaneous adipose tissue of obese human subjects and their expression profile in mouse adipose tissue and 3T3 L1 adipocytes.

GPCR Name	Log Fold change Human adipose		Log Fold change 3T3-L1	Expression in Mouse Adipose		Previously studied in adipose
	Healthy obese Vs Lean	Unhealthy obese Vs Lean	Day 7 vs Day 0	WAT	BAT	
C3AR1	1.306	3.748	-0.67	Yes	Yes	Yes
CCR5	2.391	2.494	ND	Yes	Yes	Yes
CCR7	1.51	2.377	-2.20	Yes	Yes	Yes
ADGRG3	0.876	2.258	-2.72	Yes	Yes	Yes
FPR1	0.93	2.117	-1.83	Yes	Yes	Yes
CXCR2	0.956	1.989	-2.16	Yes	Yes	Yes
CCR2	1.485	1.959	-2.10	Yes	Yes	Yes
GPR183	1.463	1.725	-1.52	Yes	Yes	Yes
CXCR4	1.053	1.702	ND	Yes	Yes	Yes
CCR1	1.596	1.67	-1.66	Yes	Yes	Yes
FPR3	1.524	1.666	ND	No	No	No
HTR7	1.611	1.655	ND	Yes	Yes	No
S1PR4	0.864	1.633	-2.53	Yes	Yes	No
ADGRE1	0.919	1.623	ND	Yes	Yes	No
CX3CR1	1.285	1.622	-1.97	Yes	Yes	Yes
GPR132	1.141	1.534	-2.08	Yes	Yes	Yes
ADORA3	1.057	1.44	ND	Trace	Trace	No
LPAR2	0.611	1.432	-1.53	Trace	Trace	Yes
CELSR1	1.058	1.379	ND	Yes	Yes	No
HRH2	0.806	1.354	-2.10	Yes	Yes	Yes
LPAR5	0.841	1.334	-1.91	Yes	Yes	No
P2RY13	0.754	1.314	-2.04	Yes	Yes	No
FPR2	0.713	1.295	-2.27	Yes	Trace	Yes
GPR85	1.038	1.253	-3.55	Yes	Yes	No
ADGRE2	0.74	1.227	ND	No	No	No
DRD1	-0.073	1.211	-1.53	Yes	trace	Yes
GPR162	0.923	1.2	-1.40	Yes	Yes	No
LHCGR	1.004	1.151	ND	Yes	Yes	No
PTAFR	0.77	1.148	ND	Yes	Yes	Yes
HRH1	0.833	1.084	-1.63	Yes	Yes	No
CMKLR1	0.809	1.047	-2.51	Yes	Yes	Yes
OPRK1	1.717	1.014	ND	No	No	Yes

3.4.1. GPRC5 Family of receptors

GPCR5 group of receptors are part of the class C GPCR family and consists of four members GPRC5A, GPRC5B, GPRC5C, and GPRC5D. These are orphan GPCRs and their expression is induced by retinoic acid. GPR5A is significantly upregulated during the adipogenesis process (Fig. 4A) and is expressed at low levels in mouse adipose tissue (Fig 4B). GPCR5A is highly expressed in human adipose and is downregulated in obese subjects (Fig4C). GPRC5B and GPRC5C are significantly upregulated during adipogenic differentiation of 3T3 L1 adipocytes (Fig 4Dand 5D) and are highly expressed in mice (Fig 4E and 4H) and human adipose tissue (Fig 4F and 4I), suggesting their possible role in adipose function. GPRC5D is expressed at trace levels in human adipose but absent in mouse adipose tissues and adipocytes (data not shown).

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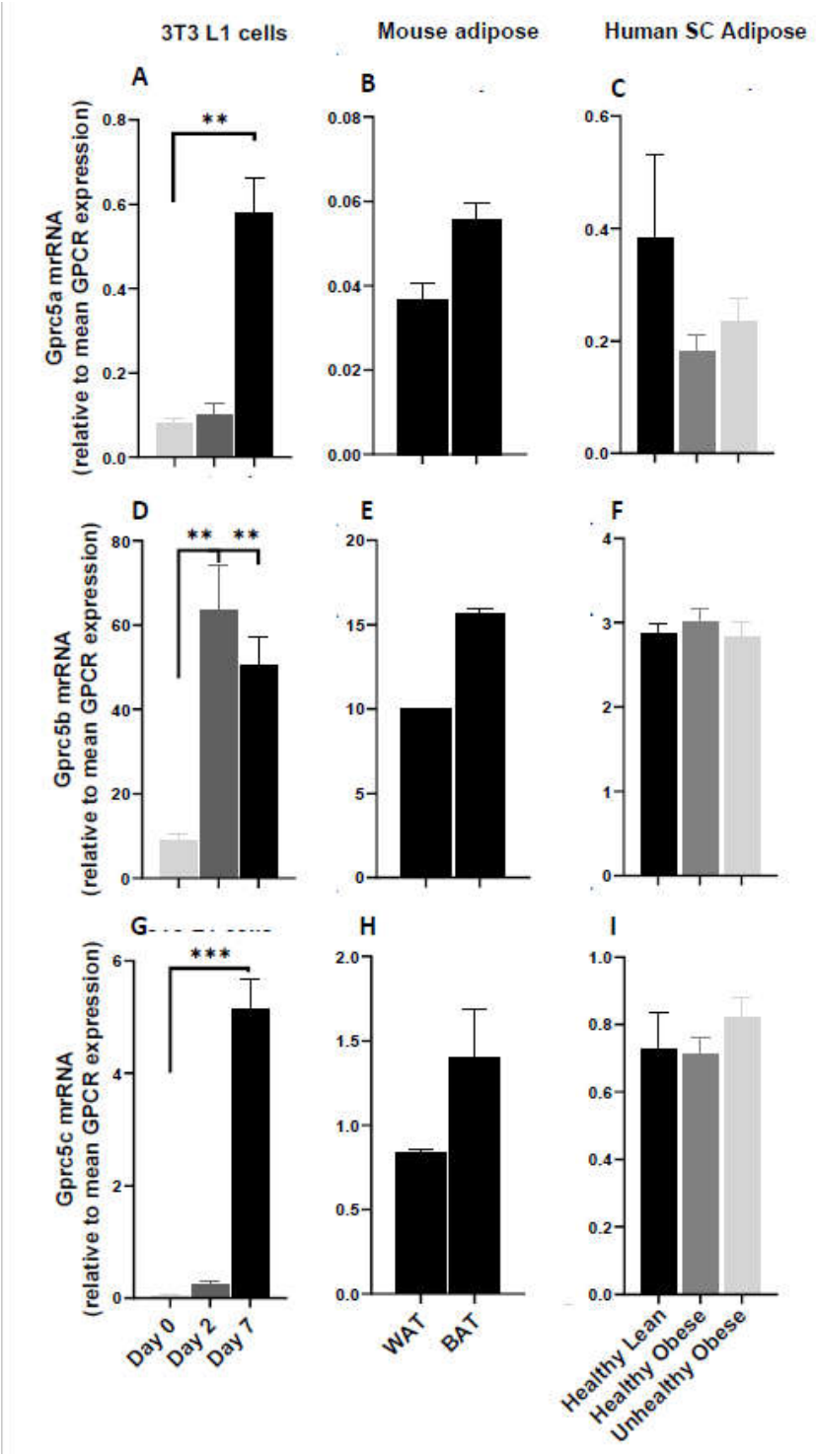

Figure 4. The mRNA expression of GPRC5 family 3T3 L1 cells, mouse adipose depots and human adipose tissue. Changes in the mRNA expression of Gprc5A, Gprc5b and Gprc5c A, D and G) during the adipogenic differentiation of 3T3 L1 cells. Each bar represents an average of three independent experiments. B, E and H) WAT and Bat from pooled tissue samples from 5 mice. Each graph represents an average of two independent experiments C, F and I) human subcutaneous adipose tissue of lean and obese human subjects. Each data point represent normalized mRNA counts from healthy lean (N=11), healthy obese (N=14) and unhealthy obese (N=20). The mRNA expression is shown relative to average mRNA expression of all detected GPCRs. Date is expressed as average ± SEM and statistical analysis by unpaired t-test. Double *Asterisk, p<0.01 and triple Asterisk, p<0.01*.

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3.4.2. G protein-coupled receptor 146 (GPR146)

GPR146 was significantly upregulated during the adipogenic differentiation of 3T3 L1 cells (Fig 5A). It was highly expressed in mouse (Fig 5B) and human adipose tissue (Fig 5C) and significantly reduced in both healthy and unhealthy obese subjects (Fig 5C). This makes GPR146 a good candidate to be studied for its possible adipose-specific role.

Fig. 5

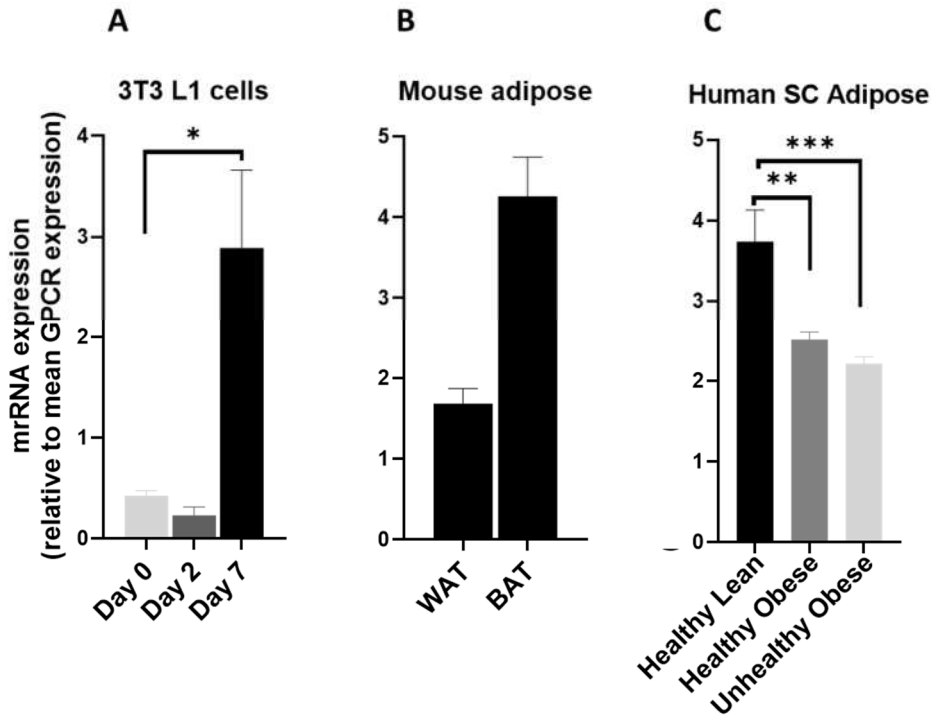

Figure 5. GPR146 mRNA in 3T3 L1 cells, mouse adipose depots and human adipose tissue. GPR146 mRNA expression A) during the adipogenic differentiation of 3T3 L1 cells. Each bar represents an average of three independent experiments B)WAT and BAT from pooled tissue samples from 5 mice. Each graph represents an average of two independent experiments C) human subcutaneous adipose tissue of lean and obese human subjects. Each data point represent normalized mRNA counts from healthy lean (N=11), healthy obese (N=14) and unhealthy obese (N=20). The mRNA expression is shown relative to average mRNA expression of all detected GPCRs. Date is expressed as average ± SEM and statistical analysis by unpaired t-test. *Asterisk, p<0.05 double Asterisk, p<0.01 and triple Asterisk, p<0.001.*

3.4.3. G protein-coupled receptor 137 (GPR137)

In this study, we observed that GPR137 is highly upregulated during the adipogenesis process (Fig. 6A). It is also highly expressed in mice (Fig 6B) and human adipose tissue (Fig 6C). These observations indicate a possible role of GPR137 in adipose tissue metabolism.

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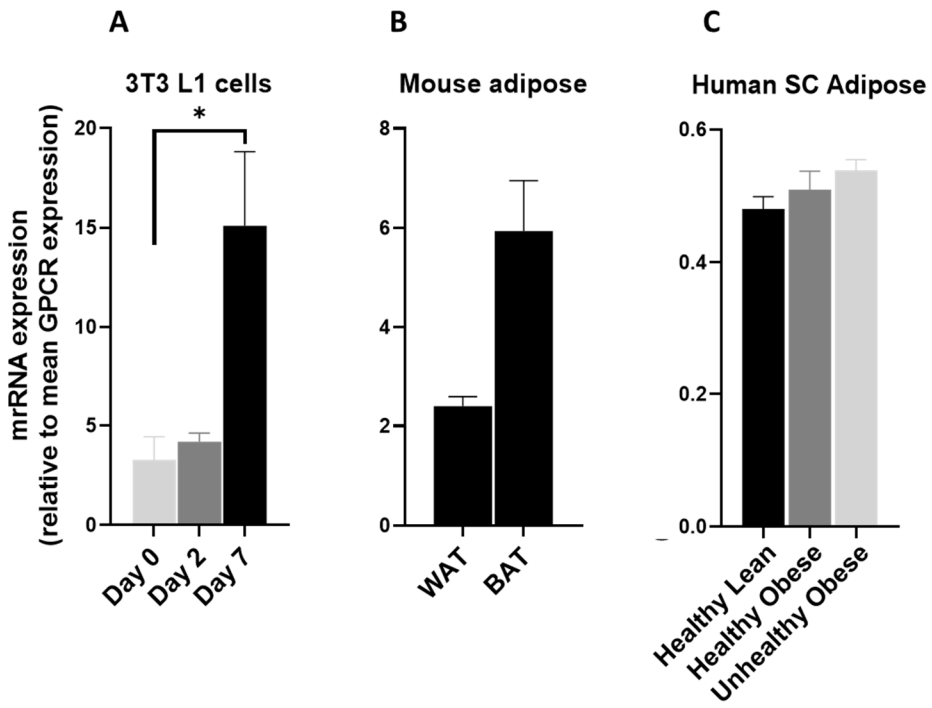

Figure 6. GPR137 mRNA in 3T3 L1 cells, mouse adipose depots and human adipose tissue. GPR137 mRNA expression A) during the dipogenic differentiation of 3T3 L1 cells. Each bar represents an average of three independent experiments B) WAT and BAT from pooled tissue samples from 5 mice. Each graph represents an average of two independent experiments C) human subcutaneous adipose tissue of lean and obese human subjects. Each data point represent normalized mRNA counts from healthy lean (N=11), healthy obese (N=14) and unhealthy obese (N=20). The mRNA expression is shown relative to average mRNA expression of all detected GPCRs. Date is expressed as average ± SEM and statistical analysis by unpaired t-test. *Asterisk, p<0.05 double Asterisk, p<0.01 and triple Asterisk, p<0.001.*

3.4.4. Arginine vasopressin receptor 1A (AVPR1A)

AVPRR1A was detected at very low levels in 3T3L1 cells (Fig 7A) and in mouse adipose tissue depots (Fig 7B) in our study. However, AVPR1A was highly expressed in human adipose tissue and was significantly downregulated in obese subjects (Fig 7C). This strongly suggests a role for AVPR1A in human adipose tissue function and possible therapeutic value.

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Fig. 7

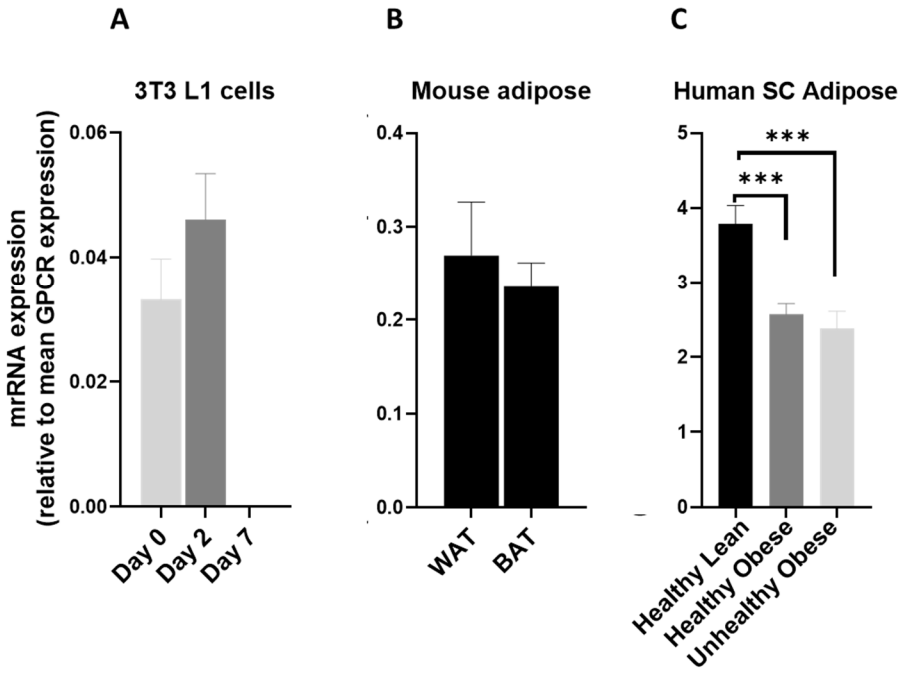

Figure 7. AVPR1A mRNA in 3T3 L1 cells, mouse adipose depots and human adipose tissue. AVPR1A mRNA expression A) during the adipogenic differentiation of 3T3 L1 cells. Each bar represents an average of three independent experiments B) WAT and BAT from pooled tissue samples from 5 mice. Each graph represents an average of two independent experiments C) human subcutaneous adipose tissue of lean and obese human subjects. Each data point represent normalized mRNA counts from healthy lean (N=11), healthy obese (N=14) and unhealthy obese (N=20). The mRNA expression is shown relative to average mRNA expression of all detected GPCRs. Date is expressed as average ± SEM and statistical analysis by unpaired t-test. *Asterisk, p<0.05 double Asterisk, p<0.01 and triple Asterisk, p<0.001.*

4. Discussion

GPCRs play a significant role in regulating adipose biology by regulating processes like adipogenesis, thermogenesis, insulin sensitivity, and glucose metabolism. However, only a few members have been comprehensively characterized and studied for their role in adipose metabolism. One of the reasons for this is the lack of information about their expression in adipose tissue and differentiating adipocytes. GPCRs are expressed at relatively lower levels and therefore, previous techniques were not sensitive enough to detect a majority of the members of this family. Previous reports have demonstrated that RNA-seq and TaqMan GPCR arrays are much better suited than transcriptomic cDNA microarrays for assessing GPCR expression (26). Due to their low abundance, Affymetrix arrays detect fewer GPCRs as compared to the Taqman arrays and RNA sequencing. We generated the expression profile of the entire set of non-visual GPCRs by using a high-throughput RT-PCR Array GPCR panel in mouse adipose tissue and differentiating adipocytes and re-analyzing RNA sequencing data of adipose tissue from healthy and obese human subjects. Many of the GPCRs with striking expression profiles have been comprehensively characterized for their adipose-specific functions and have attracted considerable attention in the drug discovery field (16-30). Chemokines and chemokine receptors have long been associated with obesity-linked inflammation and insulin resistance and modulation of the chemokine/Chemokine receptor axis is considered a novel approach for the treatment of obesity and associated metabolic disorders (31- 32). Similarly, previous studies have reported increased expression of NPYR1 expression in adipose tissue of obese human subjects and high-fat diet-fed mice (34-35). Moreover, selective antagonism of pe-

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ripheral NPYR1 was shown to prevent insulin resistance and other metabolic abnormalities in high-fat diet-fed mice (36-37). ADRβ3 and GIPR are thought to provide great therapeutic opportunities in obesity and type 2 diabetes (11-12,29). However, ADRβ3 and GIPR expression is markedly down-regulated in adipose tissue of obese human subjects in this study. This is consistent with other studies that have reported downregulation of these receptors in the adipose tissue of high fat-fed mice as well in human obese subjects (37-38). Therefore, the approach to using agonists of these receptors to mitigate obesity and related disorders is unlikely to work. This makes it even more imperative to discover newer GPCR-based targets that could offer anti-obesity therapeutic opportunities.

Our extensive analysis identified many GPCRs with striking expression profiles that merit a comprehensive functional characterization in adipose tissue metabolism. These include GPRC5 Family of receptors (GPRC5A, GPRC5B, and GPRC5C), orphan receptors GPR146 and GPR137 and Arginine vasopressin receptor 1A (AVPR1A). GPRC5B has previously been shown to activate obesity-induced inflammatory signaling in adipocytes and Gprc5b-/- mice are protected from diet-induced obesity and insulin resistance (39). In humans, GPRC5B is expressed ubiquitously and a genome-wide association study has identified GPRC5B as a genetic locus for obesity predisposition, probably due to copy-number variance (40). The functional significance of GPRC5A and GPRC5C in adipose tissue metabolism is not known and would be an exciting research subject. GPRC5A has been extensively investigated in cancer cells for its role as a tumor suppressor. In cancer cells, GPRC5A acts as a negative regulator of PI3K/AKT and cAMP/PKA pathways and it remains to be seen whether GPRC5A regulates these pathways in adipose tissue (41). GPR146 is an orphan receptor expressed widely in humans. Several studies have indicated that GPR146 is a putative receptor for C-peptide (42). Previous reports have demonstrated that GPR146 plays a vital role in the regulation of plasma cholesterol levels (43-45). A non-coding variant rs1997243 regulates blood cholesterol levels through the upregulation of GPR146 expression and GPR146-/- mice are protected from hypercholesterolemia and atherosclerosis (45). Therefore, antagonism of GPR146 has been suggested as a potential strategy to tackle atherosclerotic cardiovascular disease. The role of GPR146 in adipose tissue metabolism has not been studied. GPR137 is an orphan receptor widely expressed in human tissues and has been previously studied for its role in cancer cell growth and proliferation (46-47). GPR137 is highly expressed in several cancer types and is directly involved in cancer cell proliferation and metastasis. No previous study has investigated the role of GPR137 in adipose metabolism. AVPR1A is the receptor for arginine vasopressin (AVP) peptide and abundantly expressed in the central nervous system, heart, and liver. A wide variety of behaviors, including stress management, territorial aggressiveness, social bonding, and recognition, have been shown to be modulated by the AVP/AVPR1A signaling pathway (45-46). The AVPR1A receptors signal by activation of phospholipase C and increased intracellular calcium, which, in turn, stimulates vasoconstriction (48-50). AVPR1A expression is increased in failing hearts, and overexpression of AVPR1A in a mouse model has been shown to impair cardiac function (51). Other GPCRs with remarkable expression profiles include orphan GPCR (GPR35) (52), Prokineticin Receptor 1 (PROKR1) (53), LGR4 (54-55), and Adhesion G Protein-Coupled Receptor F5 (ADGRF5) (56). Previous studies have suggested that these GPCRs may play a critical role in controlling adipocyte biology and systemic energy homeostasis but more work is needed for a detailed understanding of their functional significance in adipose tissue metabolism. Overall, this study adds a new layer of complexity to the already intricate repertoire of genes that play crucial roles in adipose metabolism. Therefore, improving our understanding of the biological functions of such receptors in adipose tissue has clinical relevance and may prove essential in the drug discovery process

5. Conclusions

In conclusion, our comprehensive analysis identified several highly expressed GPCRs with no known role in adipose biology whose expression was significantly altered

during adipogenic differentiation and/or in the diseased human subjects. Understanding the functional importance of these GPCRs in adipose tissue metabolism might aid in the uncovering of hidden disease-drug connections and speed up medication development for obesity and metabolic diseases.

The limitations of this study include using whole adipose tissue for profiling GPCR expression. Adipose tissue is not a homogenous tissue and contains several cell types including adipocytes and a stromal vascular fraction (SVF) of cells including pre-adipocytes, fibroblasts, vascular endothelial cells, and a variety of immune cells such as adipose tissue macrophages. The cellular composition of adipose tissue may change in obesity, which may have a direct impact on the expression of genes including GPCRs. Therefore, single-cell RNA sequencing will be valuable in delineating the cell-specific expression of GPCRs in healthy and diseased states. In addition, the study relies solely on mRNA levels that may or may not reflect the changes in the protein levels. Besides the protein expression, the cellular localization of GPCRs is key to their functioning. Hence, it is important to determine the protein levels and subcellular localization of individual GPCR by immunoblotting and immunohistochemistry.

Author Contributions: S.A.M, M.O, M.A.I, G.D, A.M and M.M performed most of the experiments with assistance from S.M. J.I and S.S.M. S.M, M.R and A.Y contributed to the data analyses; S.M wrote the manuscript; S.S.M, J.I, MO and S.A.M. edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal samples were collected in according to the ethical rules of different centers.

Informed Consent Statement: Not applicable
Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: None

References

1. Pierce K. L., Premont R. T., Lefkowitz R. J. (2002). Seven-transmembrane Receptors. Nat. Rev. Mol. Cel Biol. 3, 639–650. 10.1038/nrm908
2. Strange P. G. (2008). Signaling Mechanisms of GPCR Ligands. Curr. Opin. Drug Discov. Dev. 11, 196–202
3. Pavlos N. J., Friedman P. A. (2017). GPCR Signaling and Trafficking: The Long and Short of it. Trends Endocrinol. Metab. 28, 213–226. 10.1016/j.tem.2016.10.007
4. Wang L., Zhu L., Meister J., Bone D.B., Pydi S.P., Rossi M., Wess J. Use of DREADD Technology to Identify Novel Targets for Antidiabetic Drugs. Annu. Rev. Pharmacol. Toxicol. 2021;61:421–440. doi: 10.1146/annurev-pharmtox-030220-121042.
5. Sriram K., Insel P. A. (2018). G Protein-Coupled Receptors as Targets for Approved Drugs: How many Targets and How many Drugs? Mol. Pharmacol. 93, 251–258. 10.1124/mol.117.111062
6. Hauser A.S., Attwood M.M., Rask-Andersen M., Schiöth H.B., Gloriam D.E. Trends in GPCR drug discovery: New agents, targets and indications. Nat. Rev. Drug Discov. 2017;16:829–842. doi: 10.1038/nrd.2017.178
7. Al Mahri S, Malik SS, Al Ibrahim M, Haji E, Dairi G, Mohammad S. Free Fatty Acid Receptors (FFARs) in Adipose: Physiological Role and Therapeutic Outlook. Cells. 2022 Feb 21;11(4):750. doi: 10.3390/cells11040750.
8. Amisten S, Neville M, Hawkes R, Persaud SJ, Karpe F, Salehi A. An atlas of G-protein coupled receptor expression and function in human subcutaneous adipose tissue. Pharmacol Ther. Elsevier BV; 2015;146:61–93
9. Im, H., Park, JH., Im, S. et al. Regulatory roles of G-protein coupled receptors in adipose tissue metabolism and their therapeutic potential. Arch. Pharm. Res. 44, 133–145 (2021). https://doi.org/10.1007/s12272-021-01314-w
10. Suchý T, Zieschang C, Popkova Y, Kaczmarek I, Weiner J, Liebing AD, Çakir MV, Landgraf K, Gericke M, Pospisilik JA, Körner A, Heiker JT, Dannenberger D, Schiller J, Schöneberg T, Liebscher I, Thor D. The repertoire of Adhesion G protein-coupled receptors in adipocytes and their functional relevance. Int J Obes (Lond). 2020 Oct;44(10):2124-2136. doi: 10.1038/s41366-020-0570-2. Epub 2020 Mar 19. PMID: 32203115; PMCID: PMC7508673.
11. Collins S, et al. Recent Prog Horm Res. 2001. The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. PMID: 11237219 Review.

12. Edra London, Constantine A. Stratakis, The regulation of PKA signaling in obesity and in the maintenance of metabolic health, Pharmacology & Therapeutics, Volume 237, 2022, 108113, ISSN 0163-7258, https://doi.org/10.1016/j.pharmthera.2022.108113.
13. Sheila Collins. β-Adrenoceptor Signaling Networks in Adipocytes for Recruiting Stored Fat and Energy Expenditure. Front Endocrinol (Lausanne) . 2012 Jan 3;2:102. doi: 10.3389/fendo.2011.00102.
14. Langin, D., Portillo, M. P., Saulnier-Blache, J. S. & Lafontan, M. Coexistence of three β-adrenoceptor subtypes in white fat cells of various mammalian species. Eur J Pharmacol 199(3), 291–301 (1991).
15. Atgié, C., D'Allaire, F. & Bukowiecki, L. J. Role of β1- and β3-adrenoceptors in the regulation of lipolysis and thermogenesis in rat brown adipocytes. Am J Physiol 273(4 Pt 1), C1136–42 (1997).
16. Evans BA, Merlin J, Bengtsson T, Hutchinson DS. Adrenoceptors in white, brown, and brite adipocytes. Br J Pharmacol. 2019 Jul;176(14):2416-2432. doi: 10.1111/bph.14631
17. Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M. Free Fatty Acid Receptors in Health and Disease. Physiol Rev. 2020 Jan 1;100(1):171-210. doi: 10.1152/physrev.00041.2018. Epub 2019 Sep 5. PMID: 31487233.
18. Gozal D, Qiao Z, Almendros I, Zheng J, Khalyfa A, Shimpukade B, Ulven T. Treatment with TUG891, a free fatty acid receptor 4 agonist, restores adipose tissue metabolic dysfunction following chronic sleep fragmentation in mice. Int J Obes (Lond). 2016 Jul;40(7):1143-9. doi: 10.1038/ijo.2016.37. Epub 2016 Mar 16. PMID: 26980479.
19. Meriño M, Briones L, Palma V, Herlitz K, Escudero C. Role of adenosine receptors in the adipocyte-macrophage interaction during obesity. Endocrinol Diabetes Nutr. 2017 Jun-Jul;64(6):317-327. doi: 10.1016/j.endinu.2017.03.010
20. Olaniru OE, Persaud SJ. Adhesion G-protein coupled receptors: Implications for metabolic function. Pharmacol Ther. 2019 Jun;198:123-134. doi: 10.1016/j.pharmthera.2019.02.012
21. Georgiadi A, Lopez-Salazar V, Merahbi RE, Karikari RA, Ma X, Mourão A, Klepac K, Bühler L, Alfaro AJ, Kaczmarek I, Linford A, Bosma M, Shilkova O, Ritvos O, Nakamura N, Hirose S, Lassi M, Teperino R, Machado J, Scheideler M, Dietrich A, Geerlof A, Feuchtinger A, Blutke A, Fischer K, Müller TD, Kessler K, Schöneberg T, Thor D, Hornemann S, Kruse M, Nawroth P, Pivovarova-Ramich O, Pfeiffer AFH, Sattler M, Blüher M, Herzig S. Orphan GPR116 mediates the insulin sensitizing effects of the hepatokine FNDC4 in adipose tissue. Nat Commun. 2021 May 20;12(1):2999. doi: 10.1038/s41467-021-22579-1. PMID: 34016966; PMCID: PMC8137956.
22. Wanders D, Graff EC, Judd RL. Effects of high fat diet on GPR109A and GPR81 gene expression. Biochem Biophys Res Commun. 2012 Aug 24;425(2):278-83. doi: 10.1016/j.bbrc.2012.07.082.
23. Cai TQ, Ren N, Jin L, Cheng K, Kash S, Chen R, Wright SD, Taggart AK, Waters MG. Role of GPR81 in lactate-mediated reduction of adipose lipolysis. Biochem Biophys Res Commun. 2008 Dec 19;377(3):987-91. doi: 10.1016/j.bbrc.2008.10.088. Epub 2008 Oct 24. PMID: 18952058.
24. Im H, Park JH, Im S, Han J, Kim K, Lee YH. Regulatory roles of G-protein coupled receptors in adipose tissue metabolism and their therapeutic potential. Arch Pharm Res. 2021 Feb;44(2):133-145. doi: 10.1007/s12272-021-01314-
25. Cifarelli V, Beeman SC, Smith GI, Yoshino J, Morozov D, Beals JW, Kayser BD, Watrous JD, Jain M, Patterson BW, Klein S. Decreased adipose tissue oxygenation associates with insulin resistance in individuals with obesity. J Clin Invest. 2020 Dec 1;130(12):6688-6699. doi: 10.1172/JCI141828
26. Sriram K, Wiley SZ, Moyung K, et al. Detection and Quantification of GPCR mRNA: An Assessment and Implications of Data from High-Content Methods. ACS Omega. 2019;4(16):17048-17059. Published 2019 Sep 30. doi:10.1021/acsomega.9b02811
27. Patricio Atanes, Tanyel Ashik, Shanta J. Persaud, Obesity-induced changes in human islet G protein-coupled receptor expression: Implications for metabolic regulation, Pharmacology & Therapeutics, Volume 228, 2021, 107928, ISSN 0163-7258,
28. Mohammad S, Patel RT, Bruno J, Panhwar MS, Wen J, McGraw TE. A naturally occurring GIP receptor variant undergoes enhanced agonist-induced desensitization, which impairs GIP control of adipose insulin sensitivity. Mol Cell Biol. 2014 Oct 1;34(19):3618-29. doi: 10.1128/MCB.00256-14. Epub 2014 Jul 21. PMID: 25047836; PMCID: PMC4187723.
29. Mohammad S, Ramos LS, Buck J, Levin LR, Rubino F, McGraw TE. Gastric inhibitory peptide controls adipose insulin sensitivity via activation of cAMP-response element-binding protein and p110β isoform of phosphatidylinositol 3-kinase. J Biol Chem. 2011 Dec 16;286(50):43062-70. doi: 10.1074/jbc.M111.289009.
30. Victòria Ceperuelo-Mallafré, Xavier Duran, Gisela Pachón, Kelly Roche, Lourdes Garrido-Sánchez, Nuria Vilarrasa, Francisco J. Tinahones, Vicente Vicente, Jordi Pujol, Joan Vendrell, Sonia Fernández-Veledo, Disruption of GIP/GIPR Axis in Human Adipose Tissue Is Linked to Obesity and Insulin Resistance, The Journal of Clinical Endocrinology & Metabolism, Volume 99, Issue 5, May 2014, Pages E908–E919,
31. Xue W, Fan Z, Li L, Lu J, Zhai Y, Zhao J. The chemokine system and its role in obesity. J Cell Physiol. 2019 Apr;234(4):3336-3346. doi: 10.1002/jcp.27293. Epub 2018 Oct 30. PMID: 30375006.
32. Xu L, Kitade H, Ni Y, Ota T. Roles of Chemokines and Chemokine Receptors in Obesity-Associated Insulin Resistance and Nonalcoholic Fatty Liver Disease. Biomolecules. 2015 Jul 21;5(3):1563-79. doi: 10.3390/biom5031563
33. Gericke MT, Schröder T, Kosacka J, Nowicki M, Klöting N, Spanel-Borowski K. Neuropeptide Y impairs insulin-stimulated translocation of glucose transporter 4 in 3T3-L1 adipocytes through the Y1 receptor. Mol Cell Endocrinol. 2012;348(1):27-32. doi:10.1016/j.mce.2011.07.028
34. Yang CH, Ann-Onda D, Lin X, et al. Neuropeptide Y1 receptor antagonism protects β-cells and improves glycemic control in type 2 diabetes. Mol Metab. 2022;55:101413. doi:10.1016/j.molmet.2021.101413

35. Shi Z, Bonillas AC, Wong J, Padilla SL, Brooks VL. Neuropeptide Y suppresses thermogenic and cardiovascular sympathetic nerve activity via Y1 receptors in the paraventricular nucleus and dorsomedial hypothalamus. J Neuroendocrinol. 2021;33(8):e13006. doi:10.1111/jne.13006
36. Yan C, Zeng T, Lee K, et al. Peripheral-specific Y1 receptor antagonism increases thermogenesis and protects against diet-induced obesity. Nat Commun. 2021;12(1):2622. Published 2021 May 11. doi:10.1038/s41467-021-22925-3
37. Wen-Yue Cao, Zhao Liu and Feng Guo et al. Adipocyte ADRB3 Down-Regulated in Chinese Overweight Individuals Adipocyte ADRB3 in Overweight. Obesity Facts. Vol. 11(6):524-533. DOI: 10.1159/000495116
38. Valentine JM, Ahmadian M, Keinan O, et al. β3-Adrenergic receptor downregulation leads to adipocyte catecholamine resistance in obesity. J Clin Invest. 2022;132(2):e153357. doi:10.1172/JCI153357
39. Kim YJ, Sano T, Nabetani T, Asano Y, Hirabayashi Y. GPRC5B activates obesity-associated inflammatory signaling in adipocytes. Sci Signal. 2012 Nov 20;5(251):ra85. doi: 10.1126/scisignal.2003149. PMID: 23169819.
40. Tekola-Ayele F, Lee A, Workalemahu T, Sánchez-Pozos K. Shared genetic underpinnings of childhood obesity and adult cardiometabolic diseases. Hum Genomics. 2019 Apr 4;13(1):17. doi: 10.1186/s40246-019-0202-x. PMID: 30947744; PMCID: PMC6449964.
41. Yang L, Zhao S, Zhu T, Zhang J. GPRC5A Is a Negative Regulator of the Pro-Survival PI3K/Akt Signaling Pathway in Triple-Negative Breast Cancer. Front Oncol. 2021 Feb 16;10:624493. doi: 10.3389/fonc.2020.624493.
42. Yosten GL, Kolar GR, Redlinger LJ, Samson WK. Evidence for an interaction between proinsulin C-peptide and GPR146. J Endocrinol. 2013 Jul 11;218(2):B1-8. doi: 10.1530/JOE-13-0203. PMID: 23759446.
43. Yu H, Rimbert A, Palmer AE, Toyohara T, Xia Y, Xia F, Ferreira LMR, Chen Z, Chen T, Loaiza N, Horwitz NB, Kacergis MC, Zhao L; BIOS Consortium, Soukas AA, Kuivenhoven JA, Kathiresan S, Cowan CA. GPR146 Deficiency Protects against Hypercholesterolemia and Atherosclerosis. Cell. 2019 Nov 27;179(6):1276-1288.e14. doi: 10.1016/j.cell.2019.10.034. PMID: 31778654; PMCID: PMC6889877.
44. Fernández-Ruiz I. GPR146 is a potential new therapeutic target for lipid lowering. Nat Rev Cardiol. 2020 Mar;17(3):132-133. doi: 10.1038/s41569-019-0328-5. PMID: 31848468.
45. Han F, Liu X, Chen C, Liu Y, Du M, Zhou Y, Liu Y, Song BL, He HH, Wang Y. Hypercholesterolemia risk-associated GPR146 is an orphan G-protein coupled receptor that regulates blood cholesterol levels in humans and mice. Cell Res. 2020 Apr;30(4):363-365. doi: 10.1038/s41422-020-0303-z.
46. Ren J, Pan X, Li L, Huang Y, Huang H, Gao Y, Xu H, Qu F, Chen L, Wang L, Hong Y, Cui X, Xu D. Knockdown of GPR137,G Protein-coupled receptor 137, Inhibits the Proliferation and Migration of Human Prostate Cancer Cells. Chem Biol Drug Des. 2016 May;87(5):704-13. doi: 10.1111/cbdd.12704
47. Lu J, Zhong F, Sun B, Wang C. GPR137 is a promising novel bio-marker for the prognosis of bladder cancer patients. Medicine (Baltimore). 2019;98(35):e16576. doi:10.1097/MD.0000000000016576
48. Yang SY, Kim SA, Hur GM, Park M, Park JE, Yoo HJ. Replicative genetic association study between functional polymorphisms in AVPR1A and social behavior scales of autism spectrum disorder in the Korean population. Mol Autism. 2017 Aug 9;8:44. doi: 10.1186/s13229-017-0161-9. PMID: 28808521; PMCID: PMC5550983.
49. Avinun R, Israel S, Shalev I, et al. AVPR1A variant associated with preschoolers' lower altruistic behavior. PLoS One. 2011;6(9):e25274. doi:10.1371/journal.pone.0025274
50. Fenner A. AVPR1A: a target in CRPC?. Nat Rev Urol. 2019;16(9):508. doi:10.1038/s41585-019-0218-y
51. Urbach J, Goldsmith SR. Vasopressin antagonism in heart failure: a review of the hemodynamic studies and major clinical trials. Ther Adv Cardiovasc Dis. 2021;15:1753944720977741. doi:10.1177/1753944720977741
52. Agudelo LZ, Ferreira DMS, Cervenka I, Bryzgalova G, Dadvar S, Jannig PR, Pettersson-Klein AT, Lakshmikanth T, Sustarsic EG, Porsmyr-Palmertz M, Correia JC, Izadi M, Martínez-Redondo V, Ueland PM, Midttun Ø, Gerhart-Hines Z, Brodin P, Pereira T, Berggren PO, Ruas JL. Kynurenic Acid and Gpr35 Regulate Adipose Tissue Energy Homeostasis and Inflammation. Cell Metab. 2018 Feb 6;27(2):378-392.e5. doi: 10.1016/j.cmet.2018.01.004. PMID: 29414686
53. Mok J, Park TS, Kim S, Kim D, Choi CS, Park J. Prokineticin receptor 1 ameliorates insulin resistance in skeletal muscle. FASEB J. 2021;35(2):e21179. doi:10.1096/fj.202001641R
54. Zou Y, Ning T, Shi J, Chen M, Ding L, Huang Y, Kauderer S, Xu M, Cui B, Bi Y, Liu S, Hong J, Liu R, Ning G, Wang J. Association of a gain-of-function variant in LGR4 with central obesity. Obesity (Silver Spring). 2017 Jan;25(1):252-260. doi: 10.1002/oby.21704. Epub 2016 Dec 7. PMID: 27925416.
55. Wang J, Liu R, Wang F, Hong J, Li X, Chen M, Ke Y, Zhang X, Ma Q, Wang R, Shi J, Cui B, Gu W, Zhang Y, Zhang Z, Wang W, Xia X, Liu M, Ning G. Ablation of LGR4 promotes energy expenditure by driving white-to-brown fat switch. Nat Cell Biol. 2013 Dec;15(12):1455-63. doi: 10.1038/ncb2867
56. Georgiadi A, Lopez-Salazar V, Merahbi RE, et al. Orphan GPR116 mediates the insulin sensitizing effects of the hepatokine FNDC4 in adipose tissue. Nat Commun. 2021;12(1):2999. Published 2021 May 20. doi:10.1038/s41467-021-22579-1