Original Research

Adipose *Y5R* mRNA is higher in obese than non-obese humans and is correlated with obesity parameters

Saimai Chatree, Chantacha Sitticharoon, Pailin Maikaew, Panapat Uawithya and Supornpim Chearskul

Department of Physiology, Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand
Corresponding author: Chantacha Sitticharoon. Email: chantacha.sit@mahidol.ac.th

Impact statement

Obesity, defined as excess fat accumulation, has been increasingly diagnosed worldwide causing adverse health consequences. The novel findings of this study were that *Y5R* mRNA expression in both subcutaneous and visceral fat was higher in obese than non-obese subjects. Furthermore, *Y5R* only in visceral fat, not subcutaneous fat, was positively correlated with visceral *Y1R* and obesity parameters but it was negatively correlated with the QUICKI. Moreover, we found that *Y1R* expression was highest followed by *Y5R* and *Y2R*, respectively, in both subcutaneous and visceral fat. Our results suggested that *Y5R* in visceral fat was associated with increased obesity and decreased insulin sensitivity. *Y1R* and *Y5R* might be the dominant receptors that mediate the effect of NPY-induced fat accumulation in both subcutaneous and visceral adipose tissues. *Y1R* and *Y5R* in visceral adipose tissue might be targets of drug development in prevention or treatment of obesity.

Abstract

Neuropeptide Y is mainly expressed in the central nervous system to regulate food intake via its receptors, Y receptors, and in various peripheral tissues including adipose tissue. The objectives of this study were to compare *Y5R* mRNA and adipocyte parameters consisting of area, width, height, and perimeter either between obese and non-obese subjects or between subcutaneous and visceral fat as well as to compare between *NPY*, *Y1R*, *Y2R*, and *Y5R* mRNA expressions in subcutaneous and visceral adipose tissues. In subcutaneous and visceral adipose tissues, *Y5R* was greater in obese than in non-obese humans (both $P < 0.05$). *Y1R* mRNA expression was highest followed by *Y5R*, *Y2R*, and *NPY* mRNA expressions, respectively, in subcutaneous and visceral adipose tissues. Visceral *Y5R* mRNA had positive correlations with body weight, body mass index, waist circumference, hip circumference ($R \asymp 0.4$), and visceral *Y1R* mRNA ($R = 0.773$), but had a negative correlation with the quantitative insulin sensitivity check index ($R = -0.421$) (all $P < 0.05$). Subcutaneous and visceral adipocyte parameters were positively correlated with body weight, waist circumference, hip circumference, and waist-to-hip ratio, with greater values of correlation coefficient shown in visceral ($R \asymp 0.5$–0.8) than in subcutaneous adipocytes ($R \asymp 0.4$–0.6, all $P < 0.05$). The parameters of visceral adipocytes had positive correlations with serum NPY levels ($R \asymp 0.4$, all $P < 0.05$). *Y5R* mRNA in visceral adipose tissue is related to increased obesity and reduced insulin sensitivity. The dominant Y receptors in subcutaneous and visceral adipose tissue might be the Y1R and Y5R. Visceral adipocytes show higher correlations with obesity parameters than subcutaneous adipocytes, suggestive of an increased risk of metabolic syndrome in visceral obesity. Y1R and Y5R in visceral adipose tissue might be targets of drug development in prevention or treatment of adiposity.

Keywords: Obesity, adipocyte, neuropeptide Y, Y receptor, Y5 receptor, Y1 receptor

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Introduction

Obesity is an adverse medical condition leading to a significant increase in health problems worldwide.[1] Obesity is associated with the growth of adipose tissue caused by an excess fat accumulation in adipocytes.[2] Excess adiposity is the result of adipocyte hypertrophy and/or hyperplasia.[2] There are two types of white adipose tissue depots including subcutaneous and visceral adipose tissues.[3] Subcutaneous fat is located at the hypodermis fat depot throughout the body. Visceral fat is located in the intra-abdominal compartment, including mesenteric, omental, retroperitoneal, perirenal and perigonadal regions.[3] Free fatty acids and adipokines secreted from visceral fat directly drain to the liver via the portal vein, while secretions of subcutaneous fat are transported through systemic veins.[3]

Obesity is regulated by peripheral and central signals. One of the peripheral signals, leptin, decreases expression of orexigenic neuropeptides (neuropeptide Y (NPY)/agouti-related peptide), but increases expression of anorexigenic neuropeptides (pro-opiomelanocortin/cocaine-and amphetamine-regulated transcript) in the brain, resulting in decreased food intake.[4] One of the factors regulating obesity is the 36-amino acid NPY.[4] NPY plays a central role in appetite stimulation[4] and has a peripheral action on fat proliferation[5,6] and differentiation[5] in rodents. Furthermore, it has been shown that NPY treatment decreased glycerol release in human subcutaneous adipocytes suggestive of its anti-lipolytic effect.[7] NPY binds with the Y receptor, which is a G-protein coupled receptor.[8] Seven subtypes of the Y receptor, Y1 receptor (Y1R)–Y7 receptor (Y7R), have been discovered.[9,10] Among these receptors, Y1R–Y5R are functionally expressed in humans.[11] Y1R, Y2R, and Y5R are abundantly expressed in the brain[12,13] and play a major role in feeding regulation.[14] Moreover, Y1R,[13] Y2R,[5] and Y5R[13] are also found in the periphery, such as adipose tissue. *Y5R* is the gene of interest because it is expressed in humans in the central nervous system and in peripheral adipose tissue and is involved in regulation of appetite and obesity.[8] A previous study showed that treatment of Y5R antagonist inhibited cumulative food consumption in a fasting-induced feeding model in rats.[15] Furthermore, the central blockage of Y5R function decreased body weight, fat pads, and average adipocyte area of high-energy diet-induced obese rats.[16] However, a comparison of *Y5R* mRNA expression in subcutaneous and visceral fat between obese and non-obese subjects has not been studied. This study aimed to: (1) compare *Y5R* mRNA and morphology of adipocytes between obese and non-obese subjects and between subcutaneous and visceral adipose tissues, (2) compare expressions of *NPY*, *Y1R*, *Y2R*, and *Y5R* mRNA in subcutaneous and visceral adipose tissues, and (3) determine correlations between *Y5R* mRNA or adipocyte parameters with serum NPY levels, *Y1R*, *Y2R*, and clinical characteristics. Determination of differential *NPY* and *Y* receptor expressions in different types of adipose tissues may provide information regarding which receptor subtypes should be targeted in effective management of obesity.

Material and methods

Subjects

This study was approved by the Siriraj Institutional Review Board (si533/2009) of the Faculty of Medicine Siriraj Hospital, Thailand. Informed consent was obtained from all patients. For Asian population, body mass index (BMI) $\geq$25 kg/m^2 is characterized as obese, 23–24.9 kg/m^2 as overweight, 18.5–22.9 kg/m^2 as normal weight, and $<$18.5 kg/m^2 as lean.[17] Thirty-five female subjects (20 obese, 13 non-obese, and 2 overweight) were recruited from patients undergoing abdominal surgery. Overweight subjects were included only for correlation analysis. The exclusions criteria included participants who had endocrine therapy (for instance, steroids, hormone replacement therapy, and thyroxine), pregnancy, lactation, traumatic operations, malignant diseases, endocrine diseases, and severe abdominal inflammation. Male participants could not be recruited in this study, since most of the male patients undergoing open abdominal surgery had emergency conditions or cancer. In previous studies, visceral adipose tissue collected from open abdominal surgery was obtained only in female.[13] Menstrual phase of the patients could not be controlled because most of them had myoma uteri presenting with irregular menstruation.

Demographic details and anthropometric measurements

Data on age, BMI, body weight, and waist and hip circumferences were collected. Waist circumference was measured at umbilicus level with silent breathing in the standing position. Hip circumference was measured in the standing position at inter-trochanteric girth.[18]

Tissue and blood collection

Blood samples were collected after 8 h of fasting before operations. During the operations, four to five pieces of 0.5 cm of abdominal subcutaneous and omental (visceral) adipose tissues from each patient were collected. Collected adipose tissue was snap-frozen immediately in liquid nitrogen and kept at -70°C until analysis.

Analysis of *NPY*, *Y1R*, *Y2R*, and *Y5R* mRNA expressions in adipose tissue

NPY, *Y1R*, and *Y2R* mRNA expressions were quantified as described previously.[19,20] *Y5R* mRNA was also quantified by the same technique. Briefly, total RNA was extracted by the TRIzol® reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Complementary DNA (cDNA) was synthesized by reverse transcription of 1 μg of total RNA by the iScript cDNA Synthesis Kit (Bio-RAD, Hercules, CA). Real-time polymerase chain reaction (RT-PCR) was carried out with the reagents and protocol obtained from the VeriQuest SYBR Green qPCR Master Mix (Affymetrix, Santa Clara, CA). The reference gene was *low-density lipoprotein receptor-related protein 10* (LRP10) since it was found to be the most stably expressed in adipose tissue of humans.[21] The sequences of the primers were designed and blasted to check primer specificity by the authors using nucleotide sequences published in PubMed database (Table 1). The processes of PCR amplification included (1) 95°C for 10 min for Taq DNA polymerase activation, (2) 95°C for 15 s for 40 cycles for DNA denaturing, (3) 57°C for 60 s for annealing, and (4) 72°C for 30 s for extension. For every RT-PCR reaction, a negative control was performed by no template control and positive controls were performed by using human brain and placenta tissues. A comparative procedure of quantification was calculated by the $2^{-\Delta CT}$ method.

Adipocyte morphometry

Histological staining was performed by using hematoxylin and eosin (H&E) stain to measure adipocyte morphometry. Briefly, abdominal subcutaneous and visceral adipose

Table 1. Primer sequences and product sizes.

Gene	GenBank accession numbers	Nucleotide sequences of primers	Product size (bp)
NPY[19]	NM_000905	Forward 5′-CCAGGCAGAGATATGGAAAACGA-3′ Reverse 5′-GGTCTTCAAGCCGAGTTCTGGG-3′	102
Y1R[19]	NM_000909	Forward 5′-ATCATGCTGCTCTCCATTGTGGT-3′ Reverse 5′-GTTGAAGAAGAACTGCAAGTCTCTCT-3′	222
Y2R[20]	NM_000910	Forward 5′-GGCCTACTGCTCCATCATCTTG-3′ Reverse 5′-CCCTGGGCATAGGGCACC-3′	228
Y5R	NM_006174	Forward 5′-CTGATAGCTACTGTCTGGACACT-3′ Reverse 5′-AGAGTTAAGTTGATCATCTCATTTTCTTC-3′	302
LRP10[19]	NM_014045	Forward 5′-GATGGAGGCTGAGATTGTGCA-3′ Reverse 5′-TGGAGTCATATCCTGGCGTAAG-3′	169

LRP10: lipoprotein receptor-related protein 10; Y5R: Y5 receptor; Y2R: Y2 receptor; NPY: Neuropeptide Y.

Table 2. Distribution of subjects' age, BMI, body weight, waist and hip circumference, and waist-to-hip ratio of obese and non-obese groups.

	Obese subjects ($n = 20$)			Non-obese subjects ($n = 13$)		
Clinical parameters	25th percentile	Median	75th percentile	25th percentile	Median	75th percentile
Age, year	41.3	46.0	51.0	39.5	43.0	47.0
BMI, kg/m^2	26.6	29.1	32.3	18.9	20.6	22.4
Body weight, kg	63.3	70.6	82.3	42.5	50.0	54.5
Waist circumference, cm	88.3	91.7	94.8	66.5	72.0	79.5
Hip circumference, cm	99.3	103.5	108.8	84.5	90.0	92.2
Waist-to-hip ratio	0.83	0.90	0.92	0.78	0.82	0.85

Note: Values are expressed as median and the 25th and 75th percentiles.

tissues from each patient were embedded in paraffin. Paraffin blocks were then sectioned at 20 μm thickness and paraffin slices were floated in a 40°C water bath and mounted on hydrophilic surface Twin-Mark microscope slides (Citotest Labware Manufacturing, Jiangsu, China). The sections were dried at room temperature and were kept at −70°C until staining. Adipocyte morphometry measurement was done by AxioVision® software Release 4.8.2 (Carl Zeiss AG, Oberkochen, Germany) by drawing a line overlaying the outline of the adipocyte. Width is the shortest diameter of the adipocyte, whereas height is the longest diameter of the adipocyte.

Analysis of serum NPY levels

Analysis of serum NPY levels was as described previously.[19] Briefly, levels of serum NPY were assayed by a commercial enzyme immunoassay kit (Phoenix Pharmaceuticals, Burlingame, CA). The minimum measureable concentration of NPY levels was 0.14 ng/mL and range of NPY measurement was 0–100 ng/mL. The intra-assay coefficient of variance of NPY was 10.1%.

Assessment of the quantitative insulin sensitivity check index

The quantitative insulin sensitivity check index (QUICKI) is calculated from the converse of the sum of the logarithms of fasting insulin and fasting glucose levels[22] as follows

$$1/(\log(\text{fasting insulin } \mu U/mL) + \log(\text{fasting glucose mg/dL}))$$

Statistical analysis

Data for clinical parameters are shown as median and the 25th and 75th percentiles for age, BMI, body weight, waist and hip circumferences, and waist-to-hip ratio. Data of gene expressions and adipocyte morphometry are shown as mean ± standard deviation (SD). Comparisons between obese and non-obese groups as well as between subcutaneous and visceral adipose tissues were performed by independent *t*-test. Comparisons among different gene expressions were analyzed by one-way analysis of variance followed by Fisher's least significant difference *post hoc* test. Correlation coefficient calculation was done by using the two-tailed Pearson product-moment correlation method for normally distributed data and using Spearman's Rho method for non-normally distributed data. Each multivariate linear regression model was built by stepwise analysis, incorporating the variables that showed significant correlations. The existence of high co-linearity among variables included in the study was ruled out. A P value less than 0.05 was set as statistical significance.

Results

Clinical characteristics of participants

Clinical characteristics of participants are shown as median and the 25th and 75th percentiles in Table 2. Plasma glucose, plasma insulin, and QUICKI were revealed in a previous publication showing that QUICKI was higher in non-obese compared with obese humans but plasma

glucose and insulin levels were not different between obese and non-obese groups.[23]

mRNA expressions in adipose tissue

Y5R mRNA expression was greater in the obese group than in the non-obese group ($P < 0.05$) in subcutaneous and visceral adipose tissues (Figure 1(a)). *Y5R* expression was comparable between subcutaneous and visceral fat in obese and non-obese subjects (Figure 1(b)). In obese subjects, *Y1R* showed the highest expression and was significantly higher than *Y5R*, *Y2R*, and *NPY* in both subcutaneous and visceral fat (Figure 1(c) and (d), all $P < 0.05$). *Y5R* expression was significantly higher than *Y2R* and *NPY* in visceral fat but not in subcutaneous fat (Figure 1(d), all $P < 0.05$). In the non-obese group, *Y1R* was highest expressed and significantly higher than *Y5R*, *Y2R*, and *NPY* in subcutaneous fat (Figure 1(e), all $P < 0.01$) but it was comparable with other genes in visceral fat (Figure 1(f)).

Morphology of subcutaneous and visceral adipose tissues

H&E staining showed the morphology of subcutaneous (Figure 2(a)) and visceral (Figure 2(b)) adipose tissues. Subcutaneous adipocytes had larger size than visceral adipocytes.

Adipocyte morphometry

Width and perimeter of subcutaneous adipocytes of the obese were larger than those of the non-obese group (both $P < 0.05$, Figure 2(c)). Parameters of visceral adipocytes were significantly higher in the obese than in the non-obese group (all $P < 0.01$, Figure 2(d)). Parameters of subcutaneous adipocytes were larger than those of visceral adipocytes in

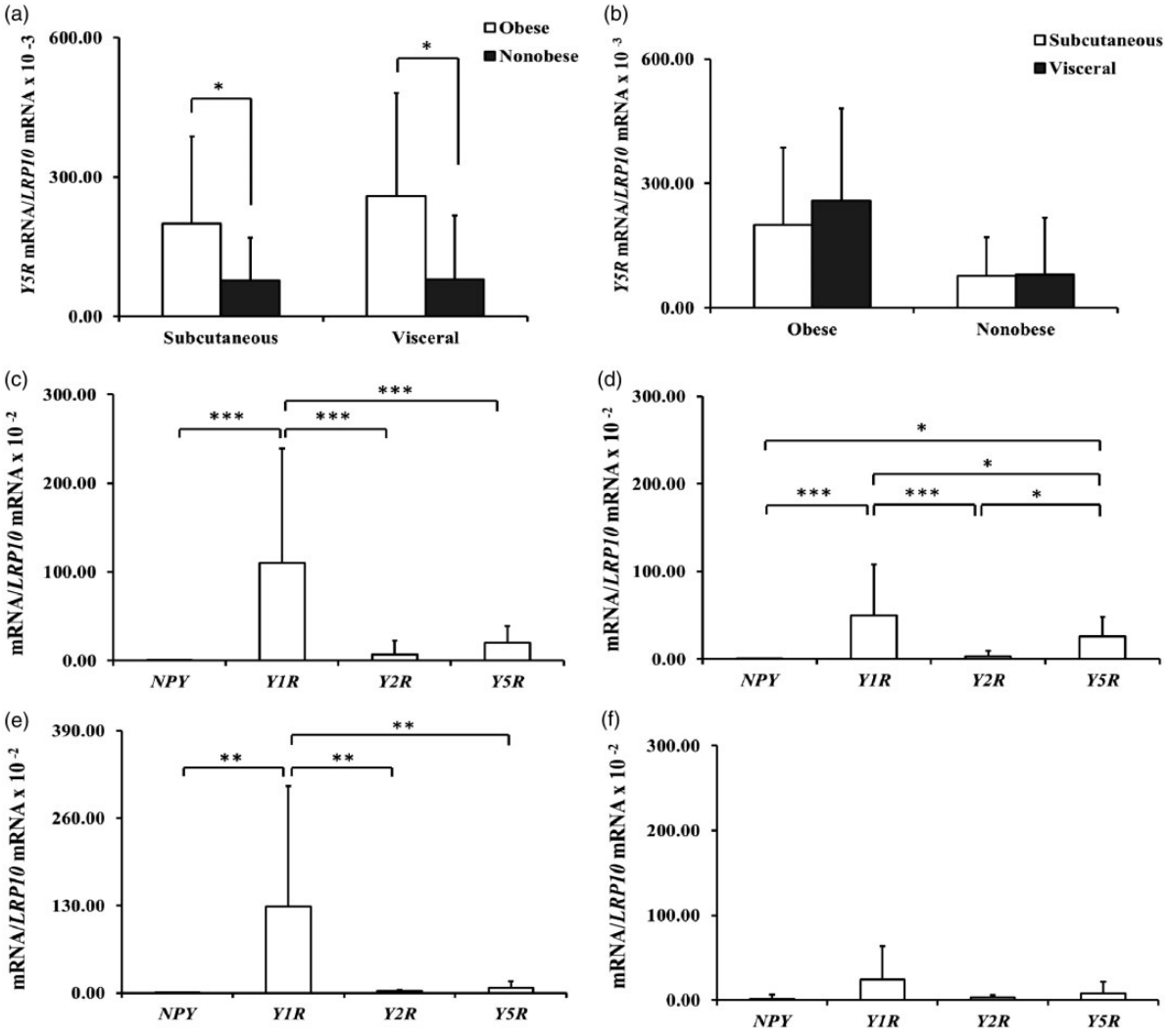

Figure 1. Mean (±SD) values of *NPY*, *Y1R*, *Y2R*, and *Y5R* mRNA expressions in subcutaneous and visceral adipose tissue specimens. Panel (a) shows *Y5R* mRNA expression compared between obese and non-obese subjects in subcutaneous and visceral fat pads. Panel (b) shows *Y5R* mRNA expression compared between subcutaneous and visceral fat pads in obese and non-obese subjects. Panels (c) and (d) show comparisons among *NPY*, *Y1R*, *Y2R*, and *Y5R* mRNA expression levels in subcutaneous and visceral adipose tissues, respectively, of obese subjects. Panels (e) and (f) show comparisons among *NPY*, *Y1R*, *Y2R*, and *Y5R* mRNA expression in subcutaneous and visceral adipose tissues, respectively, of non-obese subjects. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared between groups.

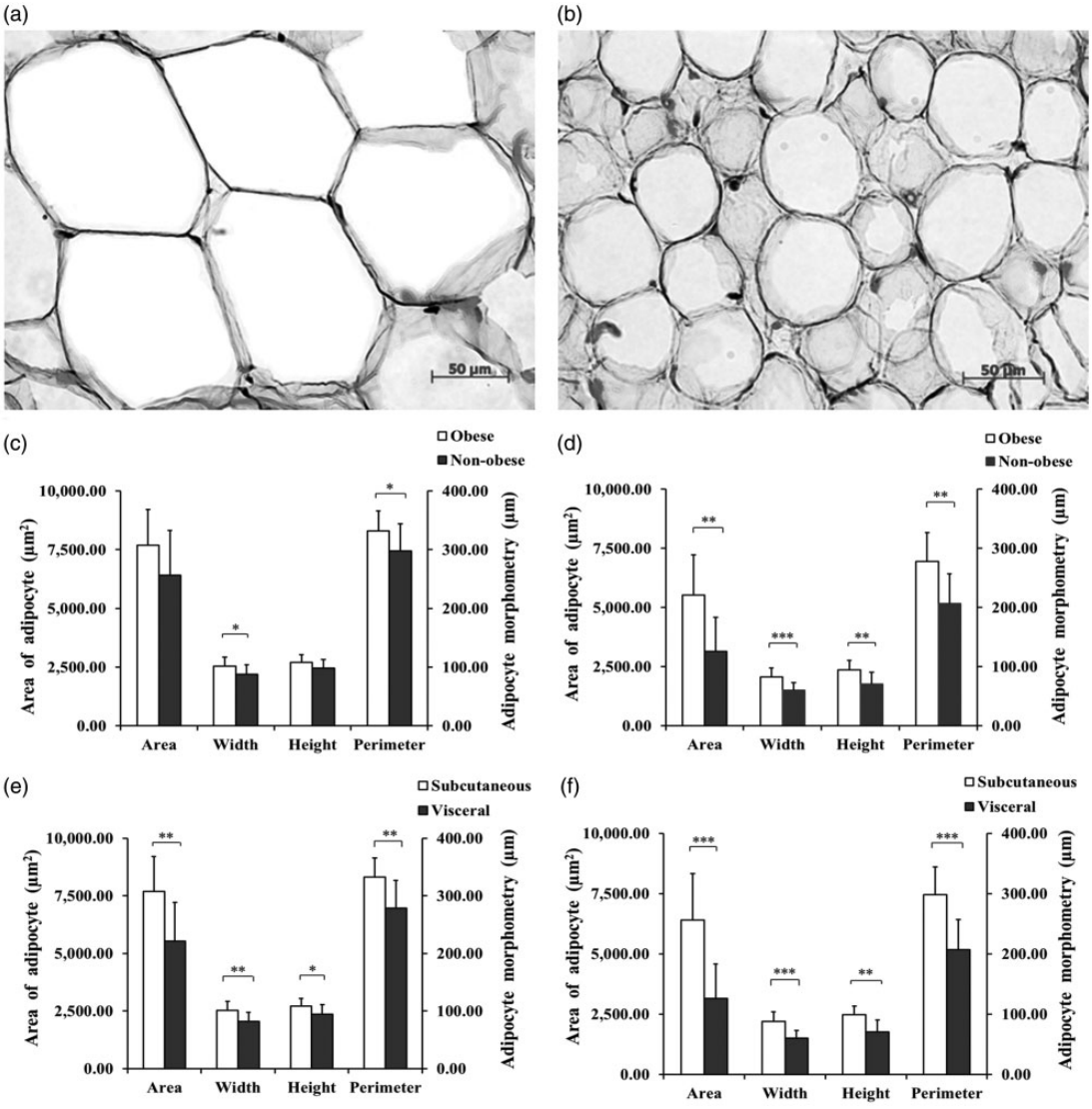

Figure 2. H&E staining and graphs showing mean (±SD) data of adipocyte characteristics, including area, width, height, and perimeter. Panels (a) and (b) show morphology of subcutaneous and visceral adipocytes, respectively. Panels (c) and (d) show area, width, height, and perimeter of subcutaneous and visceral adipocytes, respectively, compared between obese and non-obese groups. Panels (e) and (f) show area, width, height, and perimeter of adipocytes in obese and non-obese subjects, respectively, compared between subcutaneous and visceral adipocytes. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared between groups. (A color version of this figure is available in the online journal.)

obese (Figure 2(e)) and non-obese subjects (Figure 2(f), all $P < 0.05$).

Correlations between factors

Correlations between two factors are shown in Table 3. The correlation coefficient of 0–0.29 was classified as negligible correlation, 0.3–0.49 as low correlation, 0.5–0.69 as moderate correlation, 0.7–0.89 as high correlation, and 0.9–1 as very high correlation.[24] Age showed positive correlations with area, width, and perimeter of visceral adipocytes (low correlations). Body weight was positively correlated with all parameters of subcutaneous (low to moderate correlations) and visceral (high correlations) adipocytes. Height was positively correlated with area, width, and perimeter of subcutaneous adipocytes (moderate correlations) and with all parameters of visceral adipocytes (low correlations). Waist circumference was positively correlated with all parameters in visceral (moderate to high correlations) and subcutaneous (low to moderate correlations) adipocytes. Hip circumference showed positive correlations with all parameters of visceral (moderate to high correlations) and subcutaneous adipocytes (low to moderate correlations). Waist-to-hip ratio was positively correlated with area, width, and perimeter of subcutaneous adipocytes (low correlations), and with all parameters of visceral adipocytes (low to moderate correlations). There was a trend of a positive correlation between waist-to-hip ratio and height of subcutaneous adipocytes (low correlation). Moreover, serum NPY levels were positively correlated with waist circumference (low correlation), and with all parameters of visceral adipocytes (low correlations). Visceral *Y1R* expression showed a trend of a positive correlation with width of visceral adipocytes (low correlation). Subcutaneous *Y2R* expression showed a trend of negative

Table 3. Correlations between two factors.

Factors	Correlation (R)	R^2	*P*
Age – area of visceral adipocytes	0.423	0.179	<0.05*
Age – width of visceral adipocytes	0.431	0.186	<0.05*
Age – perimeter of visceral adipocytes	0.412	0.170	<0.05*
Body weight – area of subcut. adipocytes	0.593	0.352	<0.01**
Body weight – width of subcut. adipocytes	0.609	0.371	<0.01**
Body weight – height of subcut. adipocytes	0.392	0.154	<0.05*
Body weight – perimeter of subcut. adipocytes	0.610	0.372	<0.01**
Body weight – area of visceral adipocytes	0.828	0.686	<0.001***
Body weight – width of visceral adipocytes	0.804	0.646	<0.001***
Body weight – height of visceral adipocytes	0.835	0.697	<0.001***
Body weight – perimeter of visceral adipocytes	0.831	0.691	<0.001***
Height – area of subcut. adipocytes	0.560	0.314	<0.01**
Height – width of subcut. adipocytes	0.524	0.275	<0.01**
Height – perimeter of subcut. adipocytes	0.542	0.294	<0.01**
Height – area of visceral adipocytes	0.429	0.184	<0.05*
Height – width of visceral adipocytes	0.406	0.165	<0.05*
Height – height of visceral adipocytes	0.487	0.237	<0.05*
Height – perimeter of visceral adipocytes	0.419	0.176	<0.05*
Waist circumference – area of subcut. adipocytes	0.557	0.310	<0.01**
Waist circumference – width of subcut. adipocytes	0.569	0.324	<0.01**
Waist circumference – height of subcut. adipocytes	0.423	0.179	<0.01**
Waist circumference – perimeter of subcut. adipocytes	0.570	0.325	<0.01**
Waist circumference – area of visceral adipocytes	0.733	0.537	<0.001***
Waist circumference – width of visceral adipocytes	0.679	0.461	<0.001***
Waist circumference – height of visceral adipocytes	0.747	0.558	<0.001***
Waist circumference – perimeter of visceral adipocytes	0.749	0.561	<0.001***
Hip circumference – area of subcut. adipocytes	0.597	0.356	<0.01**
Hip circumference – width of subcut. adipocytes	0.520	0.270	<0.01**
Hip circumference – height of subcut. adipocytes	0.430	0.185	<0.05*
Hip circumference – perimeter of subcut. adipocytes	0.612	0.375	<0.01**
Hip circumference – area of visceral adipocytes	0.649	0.421	<0.001***
Hip circumference – width of visceral adipocytes	0.649	0.421	<0.001***
Hip circumference – height of visceral adipocytes	0.706	0.498	<0.001***
Hip circumference – perimeter of visceral adipocytes	0.660	0.436	<0.001***
Waist-to-hip ratio – area of subcut. adipocytes	0.411	0.169	<0.05*
Waist-to-hip ratio – width of subcut. adipocytes	0.461	0.213	<0.05*
Waist-to-hip ratio – height of subcut. adipocytes	0.374	0.140	0.05
Waist-to-hip ratio – perimeter of subcut. adipocytes	0.428	0.183	<0.05*
Waist-to-hip ratio – area of visceral adipocytes	0.549	0.301	<0.01**
Waist-to-hip ratio – width of visceral adipocytes	0.458	0.210	<0.05*
Waist-to-hip ratio – height of visceral adipocytes	0.526	0.277	<0.01**
Waist-to-hip ratio – perimeter of visceral adipocytes	0.567	0.321	<0.01**
Serum NPY levels – waist circumference	0.387	0.150	<0.05*
Serum NPY levels – area of visceral adipocytes	0.473	0.224	<0.05*
Serum NPY levels – width of visceral adipocytes	0.479	0.229	<0.05*
Serum NPY levels – height of visceral adipocytes	0.438	0.192	<0.05*
Serum NPY – perimeter of visceral adipocytes	0.482	0.232	<0.05*
Visceral *Y1R* mRNA – width of visceral adipocytes	0.427	0.182	0.06
Subcut. *Y2R* mRNA – area of subcut. adipocytes	−0.390	0.152	.073
Subcut. *Y2R* mRNA – perimeter of subcut. adipocytes	−0.409	0.167	.059
Visceral *Y2R* mRNA – width of subcut. adipocytes	−0.494	0.244	<0.05*
Visceral *Y2R* mRNA – width of visceral adipocytes	−0.475	0.225	<0.05*
Subcut. *Y5R* mRNA – Subcut. *Y2R* mRNA	0.437	0.191	<0.05*
Visceral *Y5R* mRNA – body weight	0.415	0.172	<0.05*
Visceral *Y5R* mRNA – BMI	0.453	0.205	<0.05*
Visceral *Y5R* mRNA – waist circumference	0.451	0.203	<0.05*
Visceral *Y5R* mRNA – hip circumference	0.495	0.245	<0.05*
Visceral *Y5R* mRNA – QUICKI	−0.421	0.177	<0.05*
Visceral *Y5R* mRNA – visceral *Y1R* mRNA	0.773	0.598	<0.001***
BMI-QUICKI	−0.534	0.285	<0.01**
Waist circumference-QUICKI	−0.628	0.394	<0.01**

subcut.: subcutaneous; Y5R: Y5 receptor; Y2R: Y2 receptor; NPY: Neuropeptide Y.
$n = 35$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

correlations with area and perimeter (low correlations) of subcutaneous adipocytes. Additionally, visceral *Y2R* expression showed significant negative correlations with width of subcutaneous and visceral adipocytes (low correlations). Subcutaneous *Y5R* expression was positively correlated with subcutaneous *Y2R* expression (low correlation). Visceral *Y5R* expression was positively correlated with body weight, BMI, waist circumference, and hip circumference (low correlations). A negative correlation was observed between visceral *Y5R* expression and the QUICKI (low correlation). Interestingly, there was a strong positive correlation between *Y5R* and *Y1R* expression in visceral adipose tissue (high correlation). QUICKI was negatively correlated with BMI and waist circumference (moderate correlations).

Multivariate regression analysis

Multivariate regression analysis showed significant associations between gene expressions and clinical parameters (Table 4). Taking visceral *Y1R* expression as the dependent variable, a significant interaction was found when visceral *Y5R* expression was an independent variable (R^2=0.597, $P < 0.001$). Subcutaneous *Y2R* expression set as the dependent variable showed a significant association with visceral *Y2R* and subcutaneous *Y5R* expressions (R^2=0.920, $P < 0.001$). Setting visceral *Y5R* expression as the dependent variable, models of significant interactions were observed by using body weight and visceral *Y1R* expression (R^2=0.676, $P < 0.001$) or BMI and visceral *Y1R* expression (R^2=0.718, $P < 0.001$) as independent variables. These two models were independently constructed to avoid co-linearity of factors that had highly significant correlations.

Discussion

This study showed that *Y5R* expression was higher in obese than in non-obese humans by 2.57-fold in subcutaneous and 3.24-fold in visceral fat. *Y5R* expression in visceral fat showed positive correlations with obesity-related parameters and visceral *Y1R*. In multiple linear regression analysis, visceral *Y5R* expression was positively correlated with body weight, BMI, and visceral *Y1R* expression. Visceral *Y1R* was positively associated with visceral *Y5R* and waist circumference. Additionally, *Y5R* was strongly and positively associated with *Y1R* in visceral fat. Our results are supported by a previous study in rodents showing that blockage of Y5R decreased body weight and mesenteric adipose tissue weight.[25] These results indicate that the Y5R has a role in increased obesity, especially in visceral adipose tissue. Furthermore, a strong positive correlation between visceral *Y5R* and visceral *Y1R* indicated that both Y receptor subtypes might work cooperatively to increase adiposity, particularly in visceral adipose tissue. This is supported by a previous study showing that there was a synergistic interaction between Y1R and Y5R in increasing food intake and body weight.[14] Furthermore, our previous study also found that *Y1R* in visceral fat was greater in obese than in normal weight humans, and was positively correlated with body weight, BMI, and waist and hip circumferences.[19] *Y5R* mRNA in visceral fat was negatively correlated with QUICKI, suggesting that visceral Y5R is related to decreased insulin sensitivity. Our result is consistent with a previous study in mice showing that a Y5R antagonist decreased plasma insulin, but not plasma glucose levels, indicating that inhibition of Y5R led to increased insulin sensitivity.[25] The action of Y5R related to decreased insulin sensitivity corresponds with that of Y1R as we previously showed that *Y1R* was positively correlated with insulin and HOMA-IR.[19] These findings suggest a role of these two receptors in increased obesity and increased insulin resistance or decrease insulin sensitivity.

In obese subjects, *Y1R* was 2058.9-fold, *Y5R* was 374.1-fold, and *Y2R* was 122.2-fold higher than *NPY* in subcutaneous fat. In visceral adipose tissue of obese subjects, *Y1R* was 245.8-fold, *Y5R* was 127.4-fold, and *Y2R* was 17.0-fold higher than *NPY*. In non-obese subjects, *Y1R* was 3645.4-fold, *Y5R* was 221.1-fold, and Y2R was 70.59-fold higher than *NPY* in subcutaneous fat. In visceral fat of non-obese subjects, *Y1R* was 14.62-fold, *Y5R* was 4.8-fold, and *Y2R* was 1.9-fold higher than NPY. These results suggest that Y1R and Y5R are dominantly expressed in human adipose tissue, especially in obese subject in both subcutaneous and visceral adipose tissues and in non-obese subjects in

Table 4. Multivariate regression analysis of gene expressions and clinical parameters.

	Model							
Dependent variable	**R**	**R^2**	***P***		**Coefficient**	**Standard error**	***t***	***P***
Visceral *Y1R* mRNA	0.773	0.597	<0.001***	(constant)	0.064	0.109	0.588	0.563
				Visceral *Y5R* mRNA	2.076	0.372	5.580	<0.001***
Subcut. *Y2R* mRNA	0.959	0.920	<0.001***	(constant)	−0.041	0.020	−2.056	0.056
				Visceral *Y2R* mRNA	2.562	0.209	12.250	<0.001***
				Subcut. *Y5R* mRNA	0.222	0.096	2.308	0.034*
Visceral *Y5R* mRNA	0.822	0.676	<0.001***	(constant)	−0.187	0.120	−1.561	0.134
				Body weight	0.005	0.002	2.206	0.039*
				Visceral *Y1R* mRNA	0.213	0.058	3.666	0.002**
Visceral *Y5R* mRNA	0.847	0.718	<0.001***	(constant)	−0.311	0.133	−2.339	0.03*
				BMI	0.016	0.006	2.923	0.008**
				Visceral *Y1R* mRNA	0.189	0.056	3.384	0.003**

subcut.: subcutaneous; Y5R: Y5 receptor; Y2R: Y2 receptor.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

subcutaneous adipose tissue. This study showed that serum NPY levels positively correlated with waist circumference and visceral adipocyte morphometry. Our previous study showed that NPY levels were higher in obese than in normal weight subjects.[19] However, NPY is produced from several peripheral tissues including adipose tissue,[6] adrenal medulla,[26] sympathetic ganglia, and tissues receiving dense sympathetic innervations.[27] Visceral *Y1R* tended to be positively correlated with width of visceral adipocytes. These results indicate that Y1R might be the major receptor subtype that determines NPY's action on increased adiposity. Blockage of Y1R might help improve the condition of obesity. Additionally, blockage of both Y1R and Y5R might help to further decrease obesity. Interestingly, in visceral fat of non-obese subjects, *Y1R* expression was not as high as that in obese subjects and was not different from *Y5R*, *Y2R*, and *NPY*. These results suggest that *Y1R* expression was remarkably increased in obese subjects resulting in a significant difference from other receptors.

Adipocyte morphometry showed a greater size in subcutaneous adipocytes than in visceral adipocytes in obese and non-obese subjects. Our results are similar to previous studies showing that the size of subcutaneous adipocytes is larger than that of visceral adipocytes in obese humans.[28] A possible explanation could be that human visceral adipocytes are more sensitive to catecholamine stimulation because of an increase in beta 1–2 adrenoceptors than subcutaneous adipocytes,[29] resulting in a decline in volume and size of adipocytes.[30] Another study reported that subcutaneous adipocytes had higher lipolytic activity than visceral adipocytes in the same amount of cells because of a larger cell volume of subcutaneous adipocytes.[31] However, the rate of lipolysis was significantly correlated to adipocyte size, regardless of either the region of origin or sex. These results indicate that lipolysis might not be the major determining factor of smaller cell size found in visceral adipocytes.

Width and perimeter, but not area and height, of subcutaneous adipocytes, and area, width, height, and perimeter of visceral adipocytes, were greater in obese than in non-obese patients. These results indicated that obese subjects had a larger size of adipocytes than non-obese patients, especially in visceral adipocytes. Our results are consistent with a previous study reporting that the adipocyte volume of abdominal subcutaneous of obese women is larger than that of non-obese women.[32] Our results showed that body weight, waist and hip circumferences, and waist-to-hip ratio were positively correlated with adipocyte morphometry in subcutaneous and visceral fat, with a much higher correlation coefficient observed in visceral fat. Our previous study revealed that visceral, but not subcutaneous, adipocyte parameters were positively correlated with insulin and HOMA-IR.[33] Taken together, obese people might have increased subcutaneous and visceral fat, with a greater increase in visceral fat, leading to increased insulin resistance and risk of metabolic syndrome.

Visceral *Y2R* mRNA was negatively correlated with width of subcutaneous and visceral adipocytes. These results suggest that Y2R is associated with a decreased adipocyte size. Our results parallel with a finding from our previous study showing that *Y2R* mRNA in visceral fat was greater in normal weight than in obese subjects.[20] These findings suggest that *Y2R* expression in adipose tissue in visceral fat might be related to adiposity reduction in humans. However, a previous study in mice showed that NPY activating through Y2R increased fat proliferation and differentiation,[5] and promoted adipogenesis *in vitro*,[34] resulting in increased body fat accumulation. The contradictory results of Y2R in regulation of obesity might be species-specific.

A positive correlation between subcutaneous *Y5R* and subcutaneous *Y2R* was observed in our study. However, our previous study showed that *Y2R* mRNA was higher in normal weight than in obese subjects in visceral, but not subcutaneous fat.[20] The correlation between subcutaneous *Y5R* and *Y2R* might not be related to adiposity, but might be related to depot-specific regulation. Depot-specific regulation of gene expression in adipose tissue was supported by our previous studies as there were positive correlations between gene expression of adipokines, including *adiponectin*, *visfatin*, *omentin*, *NPY*, and *Y* receptors, in subcutaneous or in visceral of adipose tissue, with no evidence of cross-linking between adipose tissue depots.[23,33] In multiple linear regression analysis, subcutaneous *Y2R* showed positive interactions with subcutaneous *Y5R* and visceral *Y2R*. The interaction of *Y2R* mRNA in subcutaneous and visceral fat was not related to adipose depot-specific regulation. However, this interaction might be related to its concordant action on adipose tissue as a previous study showed a high correlation coefficient ($R = 0.932$)[20] of *Y2R* between subcutaneous and visceral fat.

Age and parameters of visceral adipocytes were positively correlated, indicating that increasing age might be related to increased visceral adiposity. This result is consistent with a previous study showing that visceral fat area was positively correlated with age in sedentary subjects.[35] The increase in visceral adiposity in aging might be due to a reduction in resting metabolic rate and physical activity.[36] This study showed positive correlations between height of the subjects and parameters of subcutaneous and visceral adipocytes, with higher R values in subcutaneous than in visceral adipocytes. However, a previous study showed that height was negatively correlated with subcutaneous and visceral adipose tissue area in Chinese men.[37] This finding indicates that a larger size of adipocyte found in taller people might not be directly related to the size of adipose tissue or an indicator of determining adipose tissue mass.

Conclusion

In conclusion, this study showed that *Y5R* mRNA was higher in obese than in non-obese subjects and had positive correlations with obesity-related parameters and visceral *Y1R* gene expression. *Y1R* and *Y5R* were the major receptors in subcutaneous and visceral fat. Adipocyte morphometry of obese subjects was larger than that of non-obese subjects. Measurements of subcutaneous adipocytes were greater than those of visceral adipocytes. Visceral adipocytes showed higher correlations with body weight, waist

and hip circumferences, and waist-to-hip ratio than subcutaneous adipocytes, suggesting that increased visceral fat mass might be an indicator of an increased risk of metabolic syndrome. Collectively, the data are in agreement with previous results suggesting that Y1R and Y5R in visceral adipose tissue might have a major impact on increased obesity which might be targets of drug development in decreased adiposity. Investigation of specific blocking of Y1R and Y5R, especially in visceral adipose tissue, may reveal strategies for prevention or treatment of obesity.

Authors' contributions: All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript; S(Saimai)C and PM conducted the experiments. S(Saimai)C and CS wrote the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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