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### Title page

A pair-feeding study reveals that a Y5 antagonist causes weight loss in diet-induced obese mice by modulating food intake and energy expenditure

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Nonstandard Abbreviations: NPY, neuropeptide Y; DIO, diet-induced obesity; BAT, brown adipose tissue; WAT, white adipose tissue; ICV, intra-cerebral ventricle; MHF, moderately high fat; UCP, uncoupling protein; FFA, free fatty acid; HDL, high density lipoprotein; LDL, low density lipoprotein;  $\beta_3$ AR,  $\beta_3$ adrenergic receptor; SREBP, sterol regulatory element binding protein; Dio1, type 1 iodothyronine deiodinase; Cpt1b, muscle type carnitine palmitoyltransferase I

## **Abstract**

NPY is thought to have a significant role in the physiological control of energy homeostasis. We recently reported that an NPY Y5 antagonist inhibits body weight gain in diet-induced obese (DIO) mice, with a moderate reduction in food intake. In order to clarify the mechanism of the anti-obesity effects of the Y5 antagonist, we conducted a pair-feeding study in DIO mice. The Y5 antagonist at 100 mg/kg produced a moderate feeding suppression leading to an 18% decrease in body weight, without altering body temperature. In contrast, the pair-fed group showed only a transient weight reduction and a reduced body temperature, thus indicating that the Y5 antagonist stimulates thermogenesis. The Y5 antagonist-treated mice showed an up-regulation of uncoupling protein mRNA in brown adipose tissue (BAT) and white adipose tissue (WAT), suggesting that both BAT and WAT contribute to energy expenditure. Thus, the Y5 antagonist induces its anti-obesity effects by acting on both energy intake and expenditure.

## Introduction

Several lines of evidence have shown that the hypothalamus plays a key role in energy homeostasis, and a number of hypothalamic neuropeptides are involved in energy balance. Of these, neuropeptide Y (NPY), a 36-amino acid peptide neurotransmitter (Tatemoto et al., 1982), is one of the most potent orexigenic peptides identified. The physiological functions of NPY are mediated by activation of G-protein coupled receptors. Five NPY receptors have been cloned (Y1, Y2, Y4, Y5 and mY6) and Y1, Y2 and Y5 are abundantly expressed in the hypothalamus (Parker et al., 2002).

Accumulating evidence indicates that multiple NPY receptors regulate energy homeostasis. ICV-injected Y1 or Y5 selective agonists stimulate feeding, while chronic treatments with both classes of agonists increase body weight and adiposity (Gerald et al., 1996; Mullins et al., 2001; Mashiko et al., 2003). In contrast, intraperitoneally-injected Y2 selective agonists inhibit food intake, and exhibit anti-obesity effects (Batterham et al., 2002; Pittner et al., 2004). Since Y2 acts as an inhibitory autoreceptor, Y2 activation results in reduced NPY release, and attenuates the orexigenic effects of NPY (King et al., 1999). As expected, Y2 receptor-deficient mice exhibit an obese phenotype (Naveilhan et al., 1999). Selective Y1 receptor antagonists have been reported to suppress both NPY-induced and spontaneous food intake (Kanatani et al., 2001; Ishihara et al., 1998; Wieland et al., 1998), and NPY-induced food intake is significantly suppressed in Y1-deficient mice (Pedrazzini et al., 1998; Kanatani et al., 2000b). In addition, chronic treatment with a selective Y1 antagonist suppresses body

weight gain in Zucker fatty rats (Ishihara et al., 1998).

However, there are conflicting reports regarding the anti-obesity effects of chronic treatment with Y5 antagonists. Two Y5 antagonists, CGP71683A and GW438014A suppressed body weight gain in DIO and genetically obese models (Criscione et al., 1998; Daniels et al., 2002), while NPY5RA-971 had no effect in DIO rats (Turnbull et al., 2002). We recently demonstrated that a selective Y5 antagonist produces anti-obesity effects in mice that were developing DIO (Ishihara et al., 2006). We also demonstrated that the efficacy of the Y5 antagonist was Y5 mechanism-based using Y5 KO mice, and suggested that high and sustained brain Y5 receptor occupancy was required for the anti-obesity effects. Thus, the differing results could be explained by non-Y5 mediated off target actions, or differences in the pharmacodynamic profiles of these compounds. Interestingly, the anti-obesity effects of the Y5 antagonist are specific to the DIO model; the Y5 antagonist was not effective in genetically obese models, such as *Lep<sup>db/db</sup>* mice and Zucker fatty rats. Thus the NPY-Y5 pathway might be specifically activated, and play a key role in energy homeostasis, in DIO mice due to the high-fat feeding conditions.

In this study, we further investigated the anti-obesity mechanism of the Y5 antagonist by conducting a pair-feeding experiment in a another DIO mouse model. For these studies we used an established DIO mouse model, which had been fed a high fat diet for about 10 months. It is difficult to evaluate the precise efficacy of an anti-obesity agent in a developing DIO mouse model, since developing DIO mice show a large variation in body weight gain, due to

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differences in DIO sensitivity. In contrast, the established DIO mice show a stable obesity state, and enable us to better define the efficacy of an anti-obesity agent. Here, we show that the Y5 antagonist clearly reduced body weight in this established DIO mice model, affecting both energy intake and expenditure.

## Materials and Methods

### Materials

Y5 antagonist, 3, 3 -dimethyl- 9 - (4, 4 – dimethyl -2, 6 - dioxocyclohexyl)  
-1-oxo-1, 2, 3, 4- tetrahydroxanthene, (Kanatani et al., 2000a) was synthesized  
by Banyu Pharmaceutical Co., Ltd. (Tsukuba, Japan).

### Animals

Male C57BL/6J mice (age, 18 weeks; CLEA Japan Inc., Tokyo, Japan) were housed individually in plastic cages and were kept at  $23 \pm 2^\circ\text{C}$  and  $55 \pm 15\%$  relative humidity on a 12-h light-dark cycle (7 PM, lights off) for about 2 wk before high fat diet exposure. Water and regular chow (CE-2; CLEA Japan Inc., Tokyo, Japan) were available *ad libitum*. All experimental procedures followed the Japanese Pharmacological Society Guidelines for Animal Use.

### Experimental design

Mice were fed a moderately high-fat (MHF) diet (Oriental BioService Co., Tokyo, Japan) for about 10 months before the drug treatment started. MHF diet provides 52.4% energy as carbohydrate, 15.0% as protein, and 32.6% as fat (4.4kcal/g). MHF diet-fed mice were then divided into 3 groups, and each group was orally administered either vehicle (0.5% methylcellulose) or the Y5 antagonist at a dose of 100 mg/kg once-daily for 1 month. Dosing was performed 1.5 h before the beginning of the dark period and after measurement of daily food intake and body weight. In the pair-fed group, animals were fed

the same amount of MHF diet as that consumed by the Y5 antagonist-treated animals over the preceding 24 h. This was divided into two meals and given at 8:00 and 18:00 in order to avoid long durations of fasting. Rectal temperature was measured on the 10<sup>th</sup>, 18<sup>th</sup>, 25<sup>th</sup> and 30<sup>th</sup> days at 13:00 by insertion of thermo-probe.

After the final dosing, mice were fasted for 2 h and blood samples were collected from infraorbital veins for leptin and insulin measurement, then the mice were euthanized by collecting whole blood under isoflurane anesthesia. Plasma biochemical and lipidic parameters were measured. Liver, WAT (epididymal, retroperitoneal and mesenteric), BAT and soleus muscle were collected for measurement of mRNA expression levels, and TG contents.

### **Measurement of hormone and blood chemistry**

Plasma glucose, TG, free fatty acid (FFA), and total cholesterol (TC), HDL-cholesterol (HDL-C), and non HDL-C levels were measured using the respective commercial kits (Determiner GL-E kit and Determiner L TGII, Kyowa Medex, Japan; NEFA-HA testwako(II), Wako Pure Chemical Industries, Ltd., Japan; and Determiner L TCII, Determiner L HDL-C and Determiner L LDL-C, Kyowa Medex, Japan). Insulin and leptin levels were measured by ELISA (Morinaga, Japan). T3 and T4 were measured by RIA (Nihon Schering, Japan).

### **Measurement of TG contents**



Total lipids in the liver were extracted by the procedure of Folch et al. (Folch et al., 1957). After drying, the extracts were dissolved in isopropanol, and the TG content in the samples was measured enzymatically using a commercial kit (Determiner L TG II, Kyowa Medex).

### **Measurement of motor activity**

Another set of DIO and lean C57BL/6J mice were prepared for measurement of spontaneous motor activity. After the baseline measurement, mice were orally administered either vehicle or the Y5 antagonist at a dose of 100 mg/kg at 1.5 h before the beginning of the dark period. After the drug administration, motor activity was measured for 24 hr using an activity monitoring system (NS-AS01; Neuroscience, Tokyo, Japan). The activity monitor was composed of an infrared ray sensor placed over each cage, a signal amplification circuit, and a control unit. The sensor detected the movement of the animal on the basis of the released infrared radiation associated with its body temperature. The motor activity data were collected at 10-min intervals and analyzed with a computer-associated analyzing system (AB system-24A; Neuroscience).

### **TaqMan analysis**

TaqMan assays using an ABI Prism 7900HT sequence detector (Applied Biosystems, Foster City, CA) was performed to determine mRNA levels of uncoupling protein-1 (UCP-1), -2 (UCP-2), and -3 (UCP-3),  $\beta_3$ -adrenergic receptor ( $\beta_3$ AR) in brown (BAT) and white adipose tissue (WAT), sterol

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regulatory element binding protein-1c (SREBP1c) in liver and WAT, muscle type carnitine palmitoyltransferase I (Cpt1b) in muscle, and type 1 iodothyronine deiodinase (Dio1) in liver. Total RNA was isolated from the liver, soleus muscle, mesenteric WAT, and BAT samples using ISOGEN reagent (Nippongene, Toyama, Japan). Internal standards were adapted from 18s ribosomal RNA for liver, WAT and BAT transcripts, and beta-actin for soleus muscle transcripts. PCR reactions and analysis were performed according to the manufacturer's protocols. Sequences of primers and probes were as follows: Adrb3 (GenBank #NM\_013462), forward, CAATCCGCGCTGCTGTT, reverse, AGAAGGAGACGGAGGAGGAGA, TaqMan probe, 5'-6FAM-TTGCCTCCAACATGCCCTATGCG-TAMRA-3'; Dio1 (GenBank # NM\_007860), forward, CCAGTTCAAGAGACTCGTAGATGACT, reverse, GCGTGAGCTTCTTCAATGTAA, TaqMan probe, 5'-6FAM-TGCCTCCACAGCCGATTCCTCA-TAMRA-3'. Detailed conditions including additional sequences and fluorogenic probes for UCP-1, -2, -3, SREBP1c, and Cpt1b, were as described previously (Mashiko et al., 2003; Ito et al., 2003).

### **Statistical analysis**

Data are expressed as means  $\pm$  standard error of the mean (S.E.M.). Body weight changes were compared between groups using repeated measured two-way ANOVA coupled with a post-hoc Bonferonni/Dunn test. For food intake, blood parameters, tissue weights and mRNA levels, one-way ANOVA

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coupled with a post-hoc Bonferonni/Dunn test was performed. *P* values of <0.05 were considered significant.

## Results

### **Effects of the Y5 antagonist and pair-feeding on food intake, body weight and rectal temperature**

We administered the Y5 antagonist to DIO C57BL/6J mice that had been fed a moderately high-fat (MHF) diet for 10 months and which exhibited stable obesity with body weights of  $48.6 \pm 0.7$  g, while age-matched regular diet-fed mice weighed  $33.8 \pm 0.9$  g. Oral administration of the Y5 antagonist, at a dose of 100 mg/kg once daily, significantly reduced body weight ( $p < 0.01$ ), and food intake by about 10% ( $p < 0.01$ ), when compared with vehicle-treated mice (Fig. 1a, b). In the pair-fed group, which were fed the same amount of food as the Y5 antagonist-treated group, similar body weight reductions were initially observed ( $p < 0.01$ ). However, body weight reductions in the pair-fed group plateaued within a week, and body weight remained at a constant, reduced level during the rest of the experimental period (Fig. 1a). During this period, an approximately  $0.5^{\circ}\text{C}$  reduction in rectal temperature was observed in the pair-fed group when compared with vehicle-treated animals (Fig. 2). In contrast, Y5 antagonist-treated mice showed no reductions in rectal temperature throughout the treatment period. After 30 days of treatment, the Y5 antagonist-treated mice weighed  $41.4 \pm 0.9$  g, while the pair-fed mice weighed  $47.9 \pm 1.6$  g, the vehicle-treated mice weighed  $50.3 \pm 1.3$  g, and the regular diet-fed mice weighed  $36.7 \pm 2.6$  g.

### **Effects of the Y5 antagonist and pair-feeding on liver and adipose tissue**

### **weights and liver TG content**

At the end of drug treatment, we measured tissue weights and plasma biochemical parameters. The Y5 antagonist significantly decreased mesenteric adipose tissue weight ( $p < 0.01$ ; Fig. 3a). Similar results were observed in epididymal and retroperitoneal fat pads (data not shown). In contrast to the Y5 antagonist-treated group, no significant reduction of adipose tissue weight was observed in the pair-fed group, even though their body weight was significantly reduced. Although liver weight was not affected by treatment with the Y5 antagonist, TG content in the liver was significantly decreased ( $p < 0.05$ ; Fig. 3b, c). The pair-fed group tended to show reductions in both liver weight and TG content.

### **Effects of the Y5 antagonist and pair-feeding on plasma biochemical parameters**

Treatment with the Y5 antagonist significantly decreased plasma TC and non HDL-C levels ( $p < 0.01$ ), but did not affect plasma TG or FFA levels. These lipid parameters were not affected by pair-feeding. Treatment with the Y5 antagonist significantly reduced plasma leptin levels ( $p < 0.01$ ), while pair-feeding had no significant effect (Table 1). Plasma insulin levels were also significantly reduced in Y5 antagonist-treated mice ( $p < 0.01$ ), while the pair-fed group did not show a significant reduction (Table 1). Plasma glucose levels tended to be reduced by treatment with the Y5 antagonist, but not by pair-feeding. Pair-feeding produced a significant reduction in plasma T3 levels relative to the

vehicle-treated group ( $p < 0.05$ ), but did not produce a significant change in plasma T4 levels. In contrast, the Y5 antagonist reduced plasma T4 levels by about half ( $p < 0.01$ ), while maintaining plasma T3 levels.

### **Effects of the Y5 antagonist and pair-feeding on mRNA expression levels in white and brown adipose tissue, liver, and skeletal muscle**

In brown adipose tissue (BAT), Y5 antagonist treatment increased expression levels of uncoupling protein (UCP)-1 ( $p < 0.05$ ) and UCP-3 mRNA ( $p < 0.01$ ). Pair-feeding had no effect on these mRNA expression levels.  $\beta_3$ -adrenergic receptor ( $\beta_3$ AR) expression levels were comparable in all groups.

In white adipose tissue (WAT), treatment with the Y5 antagonist significantly increased expression levels of UCP-3,  $\beta_3$ AR and SREBP-1c mRNA levels ( $p < 0.05$ ). UCP-1 mRNA expression level tended to increase with Y5 antagonist treatment. In contrast, pair-feeding had no effect on any of these mRNA expression levels in WAT.

In liver, treatment with the Y5 antagonist significantly decreased SREBP-1c ( $p < 0.01$ ), and significantly increased type 1 iodothyronine deiodinase (Dio1) expression levels ( $p < 0.01$ ). Pair-feeding had no effect on these mRNAs expression levels.

In skeletal muscle, neither the Y5 antagonist, nor pair-feeding, had any effect on expression of UCPs or the  $\beta$ -oxidation-related gene, carnitine palmitoyltransferase-1 (Cpt1b).

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### **Effect of the Y5 antagonist on motor activity in DIO and lean mice**

We measured the motor activity to evaluate whether the prevention of hypothermia was due to the increased motor activity or not. In another set of regular chow-fed of lean mice or DIO mice, the Y5 antagonist at 100mg/kg was orally administered, and the motor activity was measured for 24hr. The Y5 antagonist did not affect motor activity either the light or the dark cycle both in lean and DIO mice (Fig. 4).

## Discussion

Chronic treatment with the Y5 antagonist significantly decreased body weight by 18%, compared to the vehicle treated group, with a moderate feeding suppression in the established DIO mice model used here. This body weight reduction lasted throughout the 30-day treatment period, and was accompanied by reductions in fat weight and improvements in plasma biochemical/hormonal parameters. In addition, TG content in liver was significantly decreased, indicating that treatment with the Y5 antagonist ameliorated obesity-related fatty liver in DIO mice. These results clearly demonstrate that the Y5 antagonist has potent anti-obesity effects in DIO mice, and corroborate our previous findings that the Y5 antagonist inhibits body weight gain during the early stages of development of DIO (Ishihara et al., 2006). In contrast, the pair-fed group showed body weight reductions only in the beginning of the treatment period. Thereafter, body weight reductions stopped, and the reduced body weight was maintained throughout the experimental period. Also in the pair-fed group, a reduced rectal temperature was observed after body weight had plateaued, and this mild hypothermia persisted throughout the experimental period. It is known that feeding suppression in rodents causes reductions in body temperature and energy expenditure, as a compensatory mechanism to conserve energy (Gavrilova et al., 1999; Severinsen and Munch, 1999). Therefore, the reduction in rectal temperature observed in the pair-fed group is thought to compensate for the reduced food intake, and may have assisted in maintaining the body weight in the pair-fed group. In contrast, reductions in rectal temperature were not



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observed in the Y5 antagonist-treated animals, despite the same reductions in food intake. This may account for the greater body weight reduction in the Y5 antagonist-treated group. In addition, the Y5 antagonist at 100mg/kg did not affect motor activity in mice. Thus, the present pair feeding study indicates that the anti-obesity effectiveness of the Y5 antagonist treatment is due to both a moderate feeding suppression, and an alteration in energy expenditure.

As target tissues for the Y5 antagonist-mediated thermogenic function, both BAT and WAT are likely to be important. Chronic treatment with the Y5 antagonist significantly increased mRNA levels of UCP-1 and UCP-3 in BAT and UCP-3 in WAT, and tended to increase UCP-1 mRNA levels in WAT, while expression of UCPs and oxidation-related genes in skeletal muscle and liver were not affected. UCP-1 is known to be a key mediator of thermogenesis, and UCP-3 is thought to be involved in thermogenesis and fatty acid oxidation (Dulloo et al., 2004). Transgenic mice that overexpress UCP-1 showed resistance to obesity (Li et al., 2000), and transgenic mice that overexpress UCP-3 had a lean phenotype (Clapham et al., 2000). Thus, the increases in UCP-1 and UCP-3 mRNA expression levels suggest that energy expenditure in BAT and WAT was elevated by chronic treatment with the Y5 antagonist. It has been reported that centrally injected NPY, or a Y5 selective agonist, decreases BAT thermogenesis (Billington et al., 1991;Hwa et al., 1999), and chronic central infusion of a selective Y5 agonist significantly decreases UCP1 mRNA expression in BAT (Mashiko et al., 2003). These data suggest that the NPY-Y5 pathway may

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modulate a thermogenic function in BAT. However, since these data were obtained after a supra-activation of the Y5 receptor, the physiological functions of the Y5 receptor to mediate energy expenditure remained undefined. Thus, the present findings obtained with the Y5 antagonist indicate a role for the Y5 receptor in energy expenditure under physiological conditions. In the present study, we demonstrated that blockade of the Y5 receptor prevented the compensatory reduction of rectal temperature, but did not increase rectal temperature above the vehicle-treated group. Since the Y5 antagonist did not increase rectal temperature above normal levels, the Y5 receptor may not alter energy expenditure during normal conditions of energy balance. The Y5 receptor may sense a negative energy balance associated with hypophagia, function to suppress energy expenditure, and prevent further weight loss. In addition to the thermogenic function of UCP-1, recently Yamada *et al* reported that ectopic expression of low levels of UCP-1 in epididymal fat decreased food intake and improved glucose tolerance in DIO mice via afferent-nerve signals or humoral factors (Yamada et al., 2006). Thus, this novel function of UCP-1 in WAT might also contribute to the anti-obesity effects of the Y5 antagonist.

The Y5 antagonist also increased  $\beta_3$ AR and SREBP-1c expression levels in WAT.  $\beta_3$ AR is the most abundant adrenergic receptor in WAT, and plays a key role in lipolysis via sympathetic stimulation. The reduction of  $\beta_3$ AR is considered to be one of the causes of DIO development. Collins *et al.* reported that  $\beta_3$ AR expression was reduced in genetic and dietary obesity in mice, and

the degree of obesity was correlated with the extent of lost  $\beta_3$ AR expression in adipocytes (Collins et al., 1997; Collins et al., 1999). Chronic treatment with the selective  $\beta_3$ AR agonist CL316,243 prevented the development of DIO, and the decline in  $\beta_3$ AR mRNA levels in WAT (Collins et al., 1997). Thus, the increase of  $\beta_3$ AR expression may produce TG mobilization in WAT, and contribute to reduced adiposity. However, the increased SREBP-1c expression level was unexpected. Since SREBP-1c is a transcriptional factor that regulates expression of genes related to lipogenesis, the increase of SREBP-1c seems to be inconsistent with reduced WAT weight. The physiological meanings of these contradictory changes are unknown at present. Perhaps there is compensatory up-regulation of SREBP-1c due to body weight reduction, or alternatively perhaps there is simultaneous activation of lipogenesis and lipolysis leading to energy dissipation within WAT. Further study will be needed to elucidate the precise functions of these changes in WAT.

Plasma T3 and T4 levels are key factors in regulating metabolic rate (Silva, 1995). In the present study, pair-feeding resulted in decreased plasma T3 levels. This is in agreement with reports showing that fasting reduces plasma thyroid hormone levels (Ahima et al., 1996). In contrast, the Y5 antagonist maintained plasma T3 levels similar to those in the vehicle-treated group. In addition, plasma T4 levels were significantly reduced in the Y5 antagonist-treated group, thus suggesting that the Y5 antagonist might stimulate the conversion of T4 to T3. In support of this observation, Dio1 mRNA levels in

liver, which is the major site of conversion outside the thyroid, were significantly increased in the Y5 antagonist-treated group when compared to the control and pair-fed groups. A similar phenomenon was reported for leptin; chronic ICV administration of leptin in rats prevented the feeding reduction-induced decrease in plasma T3 levels by stimulating conversion of T4 to T3 in liver (Cusin et al., 2000). Therefore, as was the case with leptin infusion, thyroid hormones may mediate the effects of the Y5 antagonist on energy expenditure.

We recently reported that the anti-obesity effects of the Y5 antagonist are specific to the DIO model; the antagonist was not effective in lean rodents or genetically obese models, such as *Lep<sup>db/db</sup>* mice and Zucker fatty rats (Ishihara et al., 2006). The variability in the pattern of NPY expression and its receptor in various rodent models, may account for the DIO-specific effects. A DIO rodent shows increased expression of NPY mRNA in the dorsomedial hypothalamic and ventromedial hypothalamic nuclei (Guan et al., 1998), and increased density of Y2/Y5-like receptors in hypothalamus (Widdowson et al., 1997), while genetically obese rodents showed increased expression of NPY in the arcuate nucleus (Sanacora et al., 1990) and decreased density of Y2/Y5-like receptors (Widdowson, 1997; Xin and Huang, 1998). We hypothesize that the Y5 antagonist may elicit its anti-obesity effects by improving leptin sensitivity, since the Y5 antagonist is ineffective in Zucker fatty rats and *Lep<sup>db/db</sup>* mice in which leptin function is disrupted, and thus the Y5 antagonist produces a DIO-specific effects (Ishihara et al., 2006). In support of this hypothesis, the Y5 antagonist

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produced some leptin-like peripheral metabolic effects in present study, such as increased conversion of T4 to T3, and stimulation of BAT and WAT thermogenesis. However, further investigation is necessary to elucidate the DIO-specific mechanisms of the Y5 antagonist.

In conclusion, our studies demonstrate that the Y5 antagonist affects both energy intake and expenditure to produce potent anti-obesity effects in the DIO mouse model. The Y5 antagonist may increase metabolic activity both in BAT and WAT, via neural pathways or hormonal action. The present data indicate that the NPY-Y5 receptor pathway plays a key role in energy homeostasis under pathophysiological conditions, and that a Y5 antagonist may have potential as an anti-obesity agent in humans. Indeed, very recently, Erondu *et al.*, reported that a NPY Y5 antagonist does actually produce body weight reductions in obese patients, although the magnitude of induced weight loss was not clinically meaningful (Erondu et al., 2006). Species differences in energy homeostasis, as well as different causes of obesity, may pose a significant challenge to develop effective anti-obese agents.

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## Figure legends

Fig. 1 Effects of the Y5 antagonist or pair-feeding on body weight (a) and food intake (b) in DIO mice during 30-day treatment period. The Y5 antagonist at a dose of 100mg/kg was administered once-daily. In the pair-fed group, animals were fed the same amount of diet that was consumed by the Y5 antagonist-treated animals over the preceding 24 h. Values are means  $\pm$  S.E.M. of 9-11 mice. ## P<0.01 vs. vehicle-treated *ad lib* or *pair-fed* group.

Fig. 2 Effects of the Y5 antagonist or pair-feeding on rectal temperature in DIO mice. The Y5 antagonist at a dose of 100mg/kg was administered once-daily. In the pair-fed group, animals were fed the same amount of diet that was consumed by the Y5 antagonist-treated animals over the preceding 24 h. Rectal temperature was measured on days 10, 18, 25 and 30. Values are means  $\pm$  S.E.M. of 9-11 mice # P<0.05 vs. vehicle-treated *ad lib* group.

Fig. 3 Effects of the Y5 antagonist or pair-feeding on adipose tissue (a) and liver weight (b), and liver TG content (c) in DIO mice. Values are means  $\pm$  S.E.M. of 9-11 mice # P<0.05, ## P<0.01 vs. vehicle-treated *ad lib* or *pair-fed* group.

Fig. 4 Effects of the Y5 antagonist on motor activity in C57BL/6J lean (a) and DIO (b) mice. The Y5 antagonist at a dose of 100mg/kg was administered and

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motor activity was measured for 24 hr. Motor activity was expressed as cumulative activity during light and dark cycles. Values are means  $\pm$  S.E.M. of 5-6 mice.

**Tables**

Table 1. Plasma biochemical parameters of Y5 antagonist-treated or pair-fed DIO mice.

	Vehicle	Y5 Antagonist	Pair-fed
TC (mg/dl)	156.1 ± 6.4	116.7 ± 7.8 **	136.9 ± 7.8
HDL-C (mg/dl)	123.0 ± 4.1	104.1 ± 6.6	110.4 ± 6.0
non HDL-C (mg/dl)	24.2 ± 2.0	12.2 ± 1.2 **#	19.1 ± 1.4
TG (mg/dl)	20.4 ± 1.3	25.7 ± 2.1	22.7 ± 1.9
FFA (μEq/l)	412.6 ± 23.2	456.2 ± 31.4	450.9 ± 19.1
Glucose (mg/dl)	205.5 ± 7.1	186.8 ± 10.9	195.8 ± 4.6
Insulin (ng/ml)	5.07 ± 0.50	1.74 ± 0.19 **##	4.00 ± 0.37
Leptin (ng/ml)	45.58 ± 2.63	19.25 ± 3.73**##	38.07 ± 3.36
T3 (ng/ml)	0.74 ± 0.03	0.67 ± 0.04	0.63 ± 0.03 *
T4 (μg/dl)	4.43 ± 0.17	2.02 ± 0.10 **##	4.15 ± 0.12

Data are means ± S.E.M. of 9-11 mice. \* P<0.05, \*\* P<0.01 vs. *ad lib*-fed vehicle-treated group. ## P<0.01 vs. pair-fed vehicle-treated group



Table 2. mRNA expression levels of Y5 antagonist-treated or pair-fed mice in liver, BAT, WAT and skeletal muscle.

	Vehicle	Y5 Antagonist	Pair-fed
<b>BAT</b>			
UCP-1	1.97 ± 0.22	2.75 ± 0.25 *##	1.72 ± 0.12
UCP-2	2.99 ± 0.22	3.18 ± 0.37	2.40 ± 0.23
UCP-3	0.53 ± 0.04	0.91 ± 0.05 **##	0.60 ± 0.05
β <sub>3</sub> AR	1.83 ± 0.16	1.85 ± 0.11	1.58 ± 0.14
<b>WAT</b>			
UCP-1	12.91 ± 3.25	34.68 ± 14.43	9.32 ± 4.12
UCP-2	0.56 ± 0.05	1.08 ± 0.34	0.57 ± 0.07
UCP-3	0.21 ± 0.02	0.43 ± 0.08 *	0.29 ± 0.05
β <sub>3</sub> AR	1.65 ± 0.21	3.23 ± 0.38 *#	2.03 ± 0.33
SREBP-1c	0.72 ± 0.08	1.54 ± 0.28 *	0.93 ± 0.17
<b>Liver</b>			
SREBP-1c	5.34 ± 0.50	3.30 ± 0.25 **	4.46 ± 0.41
Dio1	1.07 ± 0.07	1.54 ± 0.11**##	0.99 ± 0.11
<b>Skeletal Muscle</b>			

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UCP-2	0.60 ± 0.06	0.75 ± 0.08	0.51 ± 0.07
UCP-3	0.20 ± 0.02	0.16 ± 0.03	0.20 ± 0.02
Cpt1b	1.93 ± 0.12	1.60 ± 0.11	1.76 ± 0.12

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Data are means ± S.E.M. of 9-11 mice. \* P<0.05, \*\* P<0.01 vs. *ad lib*-fed vehicle-treated group, # P<0.05, ## P<0.01 vs. pair-fed vehicle-treated group.

Fig.1

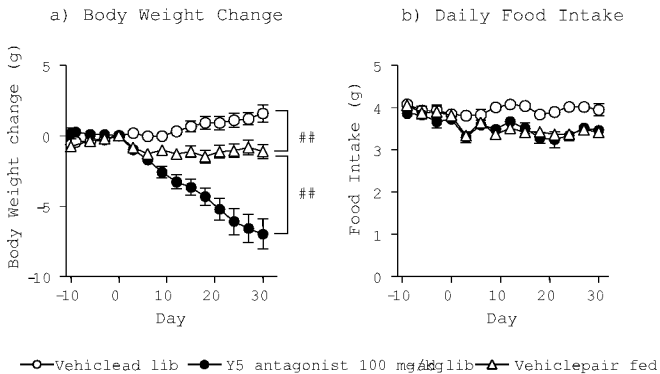


Fig.2

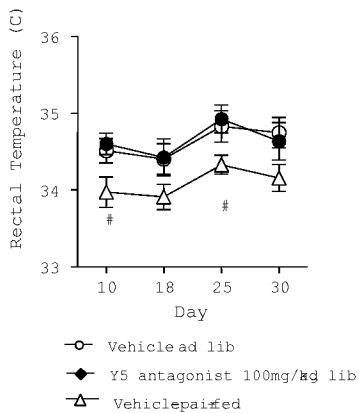


Fig. 3

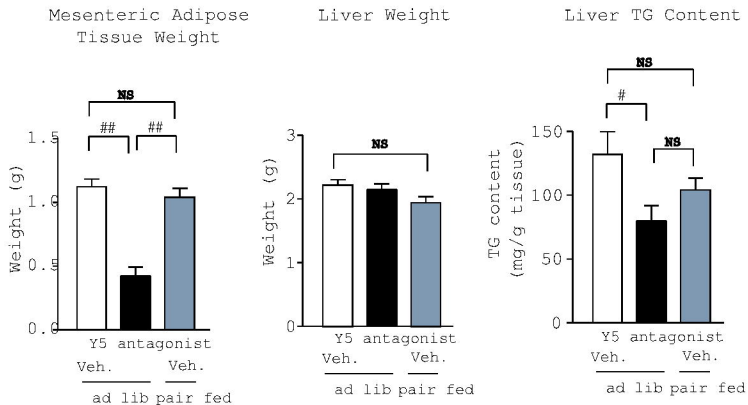
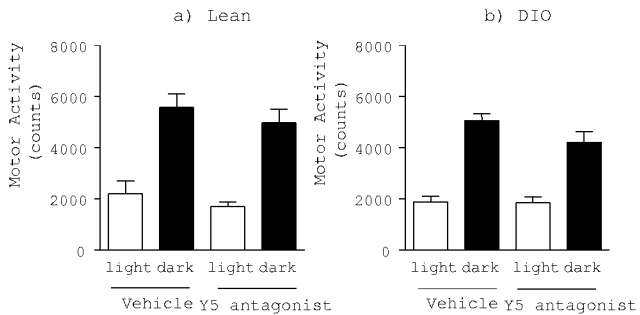


Fig. 4



# Correction to “A Pair-Feeding Study Reveals That a Y5 Antagonist Causes Weight Loss in Diet-Induced Obese Mice by Modulating Food Intake and Energy Expenditure”

Table 2 is missing from the article above [Mashiko S, Ishihara A, Iwaasa H, Sano H, Ito J, Gomori A, Oda Z, Moriya R, Matsushita H, Jitsuoka M, et al. (2007) *Mol Pharmacol* 71:602–608]. It appears in its entirety below. In addition, under the heading “Effects of the Y5 Antagonist and Pair Feeding on mRNA Expression Levels in White and Brown Adipose Tissue, Liver, and Skeletal Muscle” on page 605, text citations for Table 2 should appear after the last sentence in each paragraph.

The online version of this article will be corrected in departure from the print version.

The printer regrets this error and apologizes for any confusion or inconvenience it may have caused.

TABLE 2

mRNA expression levels of Y5 antagonist-treated or pair-fed mice in liver, BAT, WAT, and skeletal muscle

Data are means  $\pm$  S.E.M. of 9 to 11 mice.

	Vehicle	Y5 Antagonist	Pair-Fed
BAT			
UCP-1	1.97 $\pm$ 0.22	2.75 $\pm$ 0.25 <sup>***</sup>	1.72 $\pm$ 0.12
UCP-2	2.99 $\pm$ 0.22	3.18 $\pm$ 0.37	2.40 $\pm$ 0.23
UCP-3	0.53 $\pm$ 0.04	0.91 $\pm$ 0.05 <sup>***</sup>	0.60 $\pm$ 0.05
$\beta_3$ AR	1.83 $\pm$ 0.16	1.85 $\pm$ 0.11	1.58 $\pm$ 0.14
WAT			
UCP-1	12.91 $\pm$ 3.25	34.68 $\pm$ 14.43	9.32 $\pm$ 4.12
UCP-2	0.56 $\pm$ 0.05	1.08 $\pm$ 0.34	0.57 $\pm$ 0.07
UCP-3	0.21 $\pm$ 0.02	0.43 $\pm$ 0.08*	0.29 $\pm$ 0.05
$\beta_3$ AR	1.65 $\pm$ 0.21	3.23 $\pm$ 0.38 <sup>#</sup>	2.03 $\pm$ 0.33
SREBP-1c	0.72 $\pm$ 0.08	1.54 $\pm$ 0.28*	0.93 $\pm$ 0.17
Liver			
SREBP-1c	5.34 $\pm$ 0.50	3.30 $\pm$ 0.25**	4.46 $\pm$ 0.41
Dio1	1.07 $\pm$ 0.07	1.54 $\pm$ 0.11 <sup>***</sup>	0.99 $\pm$ 0.11
Skeletal muscle			
UCP-2	0.60 $\pm$ 0.06	0.75 $\pm$ 0.08	0.51 $\pm$ 0.07
UCP-3	0.20 $\pm$ 0.02	0.16 $\pm$ 0.03	0.20 $\pm$ 0.02
Cpt1b	1.93 $\pm$ 0.12	1.60 $\pm$ 0.11	1.76 $\pm$ 0.12

\*  $p < 0.05$ , \*\*  $p < 0.01$  vs. ad libitum-fed vehicle-treated group.

#  $p < 0.05$ , ##  $p < 0.01$  vs. pair-fed vehicle-treated group.