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Decreased Plasma Peptide YY Accompanied by Elevated Peptide YY and Y2 Receptor Binding Densities in the Medulla Oblongata of Diet-Induced Obese Mice

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It is well known that the peripheral peptide YY (PYY)-central neuropeptide Y (NPY) Y2 receptor axis plays an important role in promoting negative energy balance regulation. Both the hypothalamus and medulla oblongata express a high level of Y2 receptors; however, the functional role of this receptor in chronic high-fat diet-induced obesity has not been fully examined. Using quantitative autoradiography, this study measured binding densities of total [^{125}I]PYY and Y2 receptors in the hypothalamus and medulla of chronic high-fat diet-induced obese (DIO), obese-resistant, and low-fat-fed mice. Plasma PYY was also measured using RIA after 22 wk of dietary intervention. The results revealed that body weight gain was significantly higher in the obese mice, compared with the lean mice. Furthermore, PYY and NPY Y2 receptor binding densities in the medulla of the obese mice were significantly higher, compared with the lean mice, whereas the level of plasma PYY was significantly lower in the DIO mice than the low-fat-fed mice. In conclusion, this study showed that the DIO mice had low plasma PYY, which may have caused a compensatory up-regulation of PYY and Y2 receptor densities in the medulla. A low-level response of PYY-medullary regulation to positive energy balance may have contributed to the development of high-fat diet-induced obesity in DIO mice; conversely, a normal response of this regulatory axis in the obese-resistant mice may have contributed to the maintenance of body weight while on a high-fat diet. (*Endocrinology* 148: 4704–4710, 2007)

PEPTIDE YY (PYY) is a member of the pancreatic polypeptide family, which is structurally and functionally related to the neuropeptide Y (NPY) family (1, 2). PYY is mainly secreted from the endocrine L cells of the small and large intestine and released into the circulation in response to ingestion of food, especially in the presence of a fatty meal (3, 4). At present, it is known that the peripheral administration of PYY acutely inhibits food intake (5, 6). PYY has a high affinity to NPY Y2 receptors that is subsequently followed by NPY Y1 and Y5 receptors (7). Furthermore, it has been suggested that PYY works via the NPY Y2 receptor to suppress the amount of food intake (8).

In the hypothalamus, many studies have found that PYY acts on NPY Y2 receptors in the arcuate nucleus to decrease food intake (9–12). Additionally, it is known that the medulla has a high level of binding to PYY (13, 14). However, currently no information is available in regard to hypothalamic and medullary PYY and Y2 receptor regulation in diet-induced obesity. Using a chronic high-energy diet-induced obese (DIO) and diet-resistant (DR) mouse model, this study aimed to examine the levels of plasma PYY together with PYY and Y2 receptor binding density in the hypothalamus and medulla oblongata. It is hypothesized that differential regulation exists in the peripheral PYY and its hypothalamic and medullar binding densities between the mice prone or resistant to diet-induced obesity.

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Abbreviations: DIO, Diet-induced obese; DR, diet-resistant; DVC, dorsal vagal complex; IRt, intermediate reticular zone; KHT, Krebs Henseleit Tris; LF, low-fat diet control; NPY, neuropeptide Y; PYY, peptide YY; VLM, ventrolateral medulla.

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Materials and Methods

Animal model and diets

Forty-eight C57BL/6 mice aged 9 wk were obtained from the Animal Resource Centre (Perth, Western Australia) and kept in a temperature-controlled room at 22 C with a 12-h light, 12-h dark cycle. For the first week, all mice were given lab chow *ad libitum* to acclimatize them to their new surroundings. The mice were then placed in separate cages. Thirty-six mice were randomly chosen and fed a high-fat diet (Table 1) *ad libitum* and retrospectively assigned into one of three groups: DIO (n = 12), DR (n = 12), and intermediate, based on their body weight after 22 wk on this diet (15, 16). The other 12 mice were used as the control group (LF) and were given *ad libitum* low-fat diet (Table 1) for 22 wk. During the experiment, 24-h food intake and body weight were measured weekly. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and complied with the Australian Code of Practice for the Care and Use of Animal for Scientific Purposes.

Tissue preparation and body composition analysis

After 22 wk of feeding on the high- or low-fat diets, mice were given an overdose injection of sodium pentobarbitone (120 mg/kg, ip). All mice were killed between 0700 and 0900 h to minimize circadian variation. Blood samples were collected from the right ventricle of the heart. Brains were immediately removed after death and frozen in liquid nitrogen. Coronal brain sections (14 μm) were cut at −17 C using a cryostat and thaw-mounted onto polysine microscope slides (Menzel GmbH & Co. KG, Braunschweig, Germany).

Total body fat mass was measured via lipid extraction technique (17). Briefly, the mouse carcass was dried using a freeze drier (FD3 Freeze

TABLE 1. The food composition of the diet used in the experiment

	LF diet	HF diet
Cornstarch, kcal, %	67.73	38.83
Sucrose, kcal, %	6.51	5.39
Copha, kcal, %		15.75
Beef tallow, kcal, %		15.75
Sunflower oil, kcal, %	9.75	8.07
Gelatine, kcal, %	5.56	4.6
Casein, kcal, %	10.45	11.6
Fiber, g/kg	51	51
Minerals, g/kg	67	67
Vitamins, g/kg	13	13

HF, High fat; LF, low fat.

drier; Dynavac Engineering, Sydney, Australia) and weighed before and after the drying process. After that the body was cut into smaller portions then placed into cellulose thimbles. They were placed in a Soxhlet apparatus containing petroleum ether, which resulted in complete extraction of all neutral lipids. The total body fat mass was calculated by measuring the difference in body weight before and after the extraction.

[^{125}I]PYY binding autoradiography

PYY binding densities were visualized using [^{125}I]PYY as previously described (18). Sections were preincubated for 30 min in Krebs Henseleit Tris (KHT) buffer [118 mM NaCl, 4.8 mM KCl, 1.3 mM $MgSO_4$, 1.2 mM $CaCl_2$, 50 mM glucose, 15 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 10 mM Tris (pH 7.3)]. Slides were then incubated for 120 min in KHT buffer containing 0.1% BSA, 0.05% bacitracin, and 25 pM [^{125}I]PYY (Sigma Aldrich, St. Louis, MO). Nonspecific binding was determined by incubating in the same incubation buffer plus 1 μM porcine NPY (Sigma Aldrich). Slides were then washed (3 × 5 min) in ice-cold buffer, dipped in ice-cold distilled water and dried under a gentle stream of cool air. Slides were stored overnight in desiccators and then apposed to X-OMAT AR film (Kodak, Rochester, NY) in the presence of standard microscales for 12 d. Autoradiographs were developed using Kodak D-19 developer and fixed with Ilford Hypham Rapid Fixer (Ilford Imaging, Clayton, Victoria, Australia).

Y2 receptor binding autoradiography

To measure the Y2 receptor binding density, [Leu^{31}, Pro^{34}]NPY (porcine; Sigma Aldrich) was included in the incubating solution to mask the NPY Y1 and Y5 receptors (19). Briefly, sections were preincubated for 30 min in KHT buffer. Slides were then incubated for 120 min in KHT buffer containing 0.1% BSA, 0.05% bacitracin, 100 nM [Leu^{31}, Pro^{34}]NPY, and 25 pM [^{125}I]PYY (Sigma Aldrich). Porcine NPY (1 μM) was used to determine nonspecific binding as mentioned above. The remaining methods were the same as for [^{125}I]PYY binding autoradiography.

Quantification

Autoradiography images were captured and analyzed using a computer-assisted image analysis system, Multi-Analysis, connected to a GS-690 imaging densitometer (Bio-Rad Laboratories, Hercules, CA), as the detailed description was given previously (20). The density of binding was calculated with the aid of the standard curve generated from the microscales, which then converted to nanocuries per milligram of tissue equivalent. Individual medullary nuclei were identified with reference to a standard mouse brain atlas (21).

Plasma PYY

A commercially available PYY (rat, mouse, porcine) RIA kit (Phoenix Pharmaceuticals, Belmont, CA) was used to measure the plasma level of PYY. The kit had 100% cross-reactivity with both circulating forms of PYY, $PYY_{1–36}$, and $PYY_{3–36}$ (22, 23).

Statistical analysis

Data were presented as means ± SD. We used the SPSS statistical package 13.0 (SPSS Inc., Chicago, IL) for our statistical analyses. A two-way repeated ANOVA (treatment × weeks as repeated measures) was used to analyze data of the weekly body weight and energy intake. Data of PYY binding density, Y2 receptor binding density, and plasma PYY measurements were assessed by one-way ANOVA, followed by a *post hoc* Tukey-Kramer honestly significant difference test for multiple comparisons among the groups. To analyze correlations between variables measured, a Pearson test was performed.

Results

Body weight, fat mass, and energy intake

A two-way repeated ANOVA revealed significant main effects of both treatment ($F_{2,33} = 70.19$, $P < 0.001$) and the repeated measurement weeks ($F_{11,363} = 387.82$, $P < 0.001$) on weight gains. There was also a significant interaction between the two factors ($F_{22,363} = 47.13$, $P < 0.001$). Although

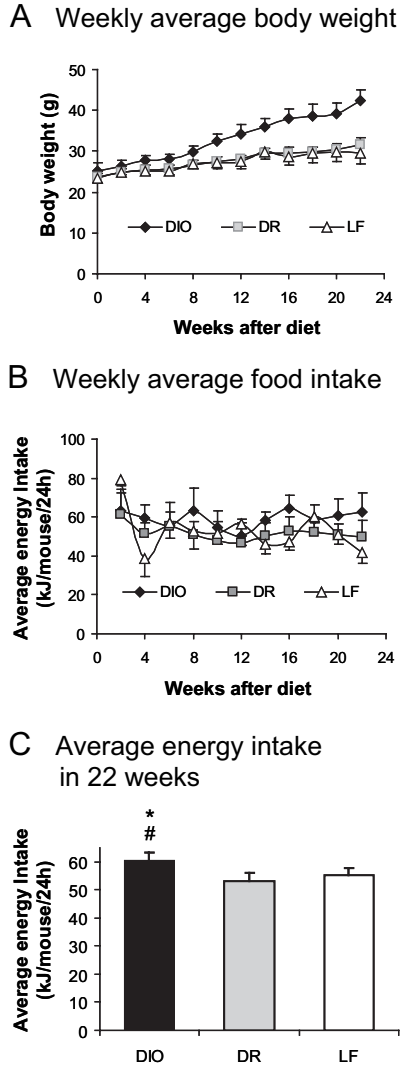

FIG. 1. Body weight (A), weekly energy intake (B), and average energy intake (C) of chronic DIO, DR, and the control (LF) mice throughout 22 wk of feeding on the high- or low-fat diet. Data are represented as mean ± SD. *Error bars* are omitted when smaller than the *symbol*. *, $P < 0.05$ DIO *vs.* LF; #, $P < 0.05$ DIO *vs.* DR.

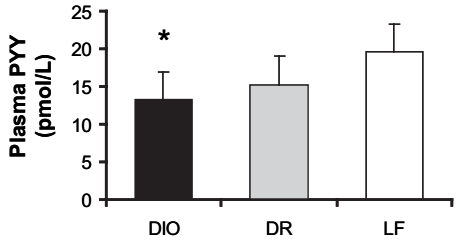

FIG. 2. The plasma level of PYY in chronic DIO, DR, and the control group (LF). Data are represented as mean ± SD. *, $P < 0.01$ *vs.* LF.

there was a consistent increase in the body weight in all groups, the DIO mice had significantly higher body weight gain than the DR ($P = 0.007$) and LF ($P = 0.001$) mice throughout the treatment period (Fig. 1A). The final body weight gain of the DIO group was 123 and 190% higher (42.7 ± 0.8 g) than the DR (31.6 ± 0.5 g) and LF mice, respectively (30.0 ± 0.8 g; $F_{2,33} = 86.54$, $P < 0.001$; DIO *vs.* DR, $P < 0.001$; DIO *vs.* LF, $P < 0.001$). Furthermore, the total fat mass of the DIO group was 129 and 192% heavier (13.3 ± 0.6 g) than the DR (6.5 ± 0.3 g) and LF mice, respectively (5.4 ± 0.4 g; $F_{2,33} = 94.4$, $P < 0.001$; DIO *vs.* DR, $P < 0.001$; DIO *vs.* LF, $P < 0.001$).

A two-way repeated ANOVA also revealed significant effects of treatment ($F_{2,33} = 19.95$, $P < 0.001$) and the repeated measurement weeks ($F_{10,330} = 15.49$, $P < 0.001$) on food intake along with a significant interaction between the two factors ($F_{20,30} = 7.94$, $P < 0.001$). Throughout the treatment period, the DIO mice had a significantly higher energy intake, compared with DR mice ($P < 0.001$; Fig. 1B). Although there was a fluctuation in the energy intake of the LF mice on the various measurement days, overall, the DIO mice had a higher energy intake than the LF mice ($P < 0.001$; Fig. 1B). No significant differences were found between the DR and LF mice. Furthermore, the average energy intake throughout the 22 wk of feeding was significantly higher in the DIO group (60.49 ± 0.87 kJ per 24 h), compared with the DR (53.03 ± 0.91 kJ per 24 h) and LF groups (55.13 ± 0.71 kJ per 24 h; $F_{2,33} = 21.31$, $P < 0.001$; DIO *vs.* DR, $P < 0.001$; DIO *vs.* LF, $P < 0.001$; Fig. 1C).

Plasma PYY

The level of plasma PYY was significantly lower in the DIO group (32%), compared with the LF group ($F_{2,21} = 6.26$, $P < 0.01$; DIO *vs.* LF, $P = 0.007$; Fig. 2). No significant difference was found in the levels of plasma PYY between the DR and LF groups.

PYY binding density and Y2 receptor binding density in the hypothalamus of DIO, DR, and LF mice

Although there was a trend that the obese mice had a higher PYY binding density, compared with the lean mice, in the dorsomedial and ventromedial hypothalamus, these differences were not significant (Table 2). There was not any significant difference in PYY binding density in the arcuate nucleus and lateral hypothalamus. Furthermore, there were no significant differences of Y2 receptor binding density in any hypothalamic nuclei (Table 2) between the groups.

PYY binding density in the medulla of DIO, DR, and LF mice

A one-way ANOVA revealed that there were significant differences between the groups for PYY binding density in the dorsal vagal complex (DVC) containing the nucleus of solitary tract and dorsal motor nucleus of vagus nerve ($F_{2,10} = 6.76$, $P = 0.019$), intermediate reticular zone (IRt; $F_{2,10} = 17.34$, $P = 0.001$), and ventrolateral medulla (VLM; $F_{2,10} = 7.60$, $P = 0.014$) area (Figs. 3 and 4). In the DVC, the DIO mice had higher PYY binding density than that of the DR (68%, $P = 0.015$) and LF mice (37%, $P = 0.079$). Similar differences were also observed in the intermediate reticular zone, in which the DIO had a significantly higher binding density, compared with the DR (171%, $P = 0.001$) and the LF group (96%; $P = 0.004$). In the VLM, the DIO mice also had a significantly higher binding density than the DR (122%, $P = 0.022$) and the LF group (134%, $P = 0.015$).

Y2 receptor density in the medulla oblongata of DIO, DR, and LF mice

The DIO mice had significantly higher Y2 receptor binding density (Figs. 3 and 4), in the DVC, compared with the LF group (63%; $F_{2,10} = 4.75$, $P = 0.044$; DIO *vs.* LF, $P = 0.041$). In the IRt, the DIO group had 47% higher binding density, compared with the DR group ($F_{2,10} = 6.92$, $P = 0.02$; DIO *vs.* DR, $P = 0.023$). In the VLM, there were no differences in the binding density among the DIO, DR, and LF groups.

TABLE 2. The [^{125}I]PYY binding and Y2 receptor binding densities in various areas of the hypothalamus of chronic DIO, DR, and the control (LF) mice

Brain area	DIO[a]	DR[a]	LF[a]	F (2, 10)	*P* value
Total binding with [^{125}I]PYY					
Arc	284.8 ± 0.7	198.6 ± 66.5	195.7 ± 38.5	3.00	0.160
DMH	213.1 ± 27.7	123.7 ± 44.7	172.0 ± 20.9	4.39	0.079
LH	224.6 ± 30.1	131.2 ± 46.1	177.9 ± 42.7	3.02	0.138
VMH	248.60 ± 49.3	138.6 ± 41.4	168.3 ± 27.9	5.02	0.064
Y2 receptor binding density					
Arc	228.5 ± 17.2	154.3 ± 70.2	170.9 ± 35.5	1.36	0.337
DMH	206.2 ± 16.2	125.1 ± 58.9	154.3 ± 37.4	1.98	0.232
LH	222.1 ± 5.3	131.2 ± 51.0	162.6 ± 42.4	2.83	0.151
VMH	189.5 ± 30.9	139.9 ± 38.2	143.4 ± 23.7	1.72	0.269

Binding densities were quantified at the level of bregma −1.22, −1.70, and −2.18 mm. Arc, Arcuate nucleus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus.

[a] Mean ± SD.

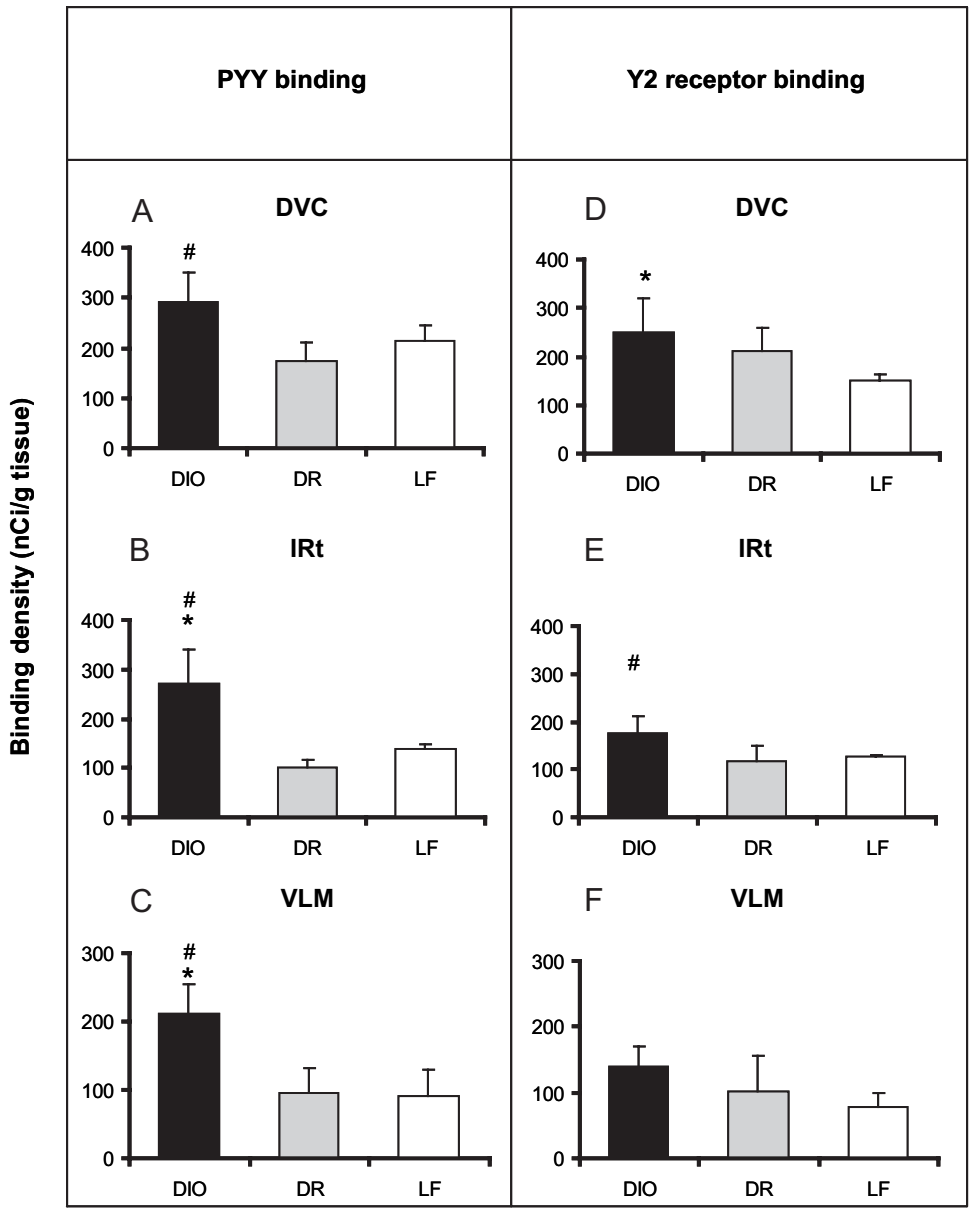

FIG. 3. The binding densities to [^{125}I]PYY (A–C) and Y2 receptor (D–F) in the DVC, IRt, and VLM of chronic DIO, DR, and the control (LF) mice. Binding densities were quantified at the level of bregma −7.32. Data are represented as mean ± SD. *, $P < 0.05$, DIO *vs.* LF; #, $P < 0.05$ DIO *vs.* DR.

Correlation

A correlation analysis was carried out among the final body weight, body fat mass, energy intake of the last week of the dietary intervention, and the plasma PYY as well as the medullary PYY binding density and Y2 receptor binding density of the DIO, DR, and LF mice (Table 3). Final body weight was highly correlated to the plasma PYY and the PYY binding densities in all measured areas as well as the Y2 receptor binding densities in the IRt and VLM. Total body fat was also highly correlated to the plasma PYY, the PYY binding densities in all measured areas, and the Y2 receptor binding density in the IRt and the VLM. The energy intake of the last week was significantly correlated to plasma PYY concentrations and the PYY binding densities and Y2 receptor binding densities in the IRt and the VLM.

Discussion

The results of this study revealed a significant increase of medullary PYY and Y2 receptor binding densities in the DIO mice, compared with the DR and LF mice. This increased binding density was accompanied by a decrease in plasma PYY level.

It is known that PYY acts on NPY Y2 receptors in the hypothalamic arcuate nucleus to decrease food intake (9–12). Furthermore, Y2 receptor conditional knockout mice have been shown to have a significant increase of food intake (24). Although there is an abundant amount of NPY receptor in the medulla (25, 26), to our present knowledge, no information is available in respect to whether the medullary NPY receptors are involved in the regulation of body weight in high-fat diet-induced obesity.

This study found that the PYY and Y2 receptor binding

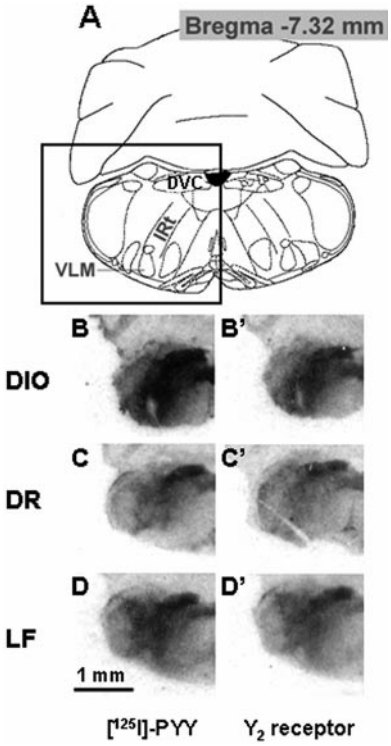

FIG. 4. Photographs depicting the [^{125}I]PYY bindings (B–D) and Y2 receptor bindings (B' to D') in the medulla of chronic DIO (B and B'), DR (C and C'), and the LF (control) group (D and D'). The *line box* (A) indicates where the section was taken.

densities in the obese mice were significantly higher than the lean DR and LF mice in most areas of the medulla regulating autonomic function (DVC, IRt, and VLM). This study also found that there was a positive correlation between PYY binding density in these areas and final body weight, energy intake, and body fat mass. Furthermore, although a similar trend was found in some of the hypothalamic nuclei (the dorsomedial and ventromedial hypothalamus), the differences between the obese and lean mice were not as significant as the findings in the medullary areas. These findings have demonstrated that PYY possibly acts to regulate energy balance via the medulla to control food intake rather than working exclusively in the arcuate hypothalamic nucleus.

The differences in the binding density in the VLM implied that there might be a difference between the obese and lean mice in their baroreceptor regulation by PYY (27). This is supported by the finding that the VLM is a site that plays a crucial role in baroreceptor regulation in hypertensive obese rats (28). In general, there is a tendency for obese subjects to have higher blood pressure (29–31). However, to our present knowledge, there is no literature available that evaluates the baroreceptor regulation in a diet-induced obese mouse model.

Studies have shown that an injection of PYY into the DVC causes similar neuronal inhibition as an injection of a Y2 agonist (13). This implies that PYY binds to Y2 receptors in the medulla to cause these effects. In the present study, the obese mice were found to have a significantly higher Y2 receptor binding density in the DVC and IRt areas of the medulla, compared with the lean mice. Because the magnitude of the significance in the correlation between Y2 receptor binding density and body weight, food intake, and body fat mass was less than that of PYY binding density, it is possible that the mechanism in which the PYY regulates the food intake in the medulla might be not only acting solely on the NPY Y2 receptor binding density as previously thought but may also by acting through the NPY Y1 and Y5 receptors.

Plasma PYY in the obese mice in this study was found to be lower than the high-fat-fed lean and the low-fat-fed lean mice. Other studies in humans and rodents have also described a reduced amount of plasma PYY in obese populations (32, 33). This attenuated response of PYY release in obese populations (34) has been shown to cause an insufficient inhibitory effect on feeding (35, 36). Findings from the analysis of PYY null mice also confirm that a depleted amount of PYY can cause an increase in food intake (37). This study is the first to analyze the peripheral plasma PYY as well as its receptor binding density in a dietary-induced obese animal model. Based on our results, we suggest that the up-regulation of the medullary PYY binding density in the DIO mice may be a response to their low level of plasma PYY. However, it is obvious that in the diet-induced obese mice, this compensatory regulation of PYY was not effective enough to reduce the food intake in this group. This is possibly due to the low amount of PYY bound as a result of the low plasma PYY the obese mice had. It is important to note that PYY-immunoreactive neurons have been found in the medulla (38, 39). Therefore,

TABLE 3. The correlation among the body weight, food intake, and plasma level of PYY and binding densities of [^{125}I]PYY and Y2 receptor in the medulla nuclei

	After 22 wk of high-fat diet					
	Final body weight		Total body fat		Energy intake in last week	
	R value	*P* value	R value	*P* value	R value	*P* value
Plasma PYY	−0.468	0.021	−0.475	0.019	−0.462	0.023
Total binding with [^{125}I]PYY						
DVC	0.738	0.010	0.738	0.010	0.516	0.104
IRt	0.842	0.001	0.838	0.001	0.671	0.024
VLM	0.803	0.003	0.817	0.002	0.777	0.005
Y2 receptor binding density						
DVC	0.495	0.122	0.468	0.146	0.431	0.186
IRt	0.727	0.011	0.747	0.008	0.767	0.006
VLM	0.618	0.043	0.634	0.036	0.841	0.001

a local effect of PYY neurons on PYY binding and Y2 receptor binding cannot be excluded. Further studies are needed to confirm this issue by measuring the levels of PYY mRNA and protein expression in the medulla of DIO mice.

Furthermore, the results of this study suggested that the elevation of the level of plasma PYY might be effective at decreasing food intake. However, previous studies have shown that there were different effects of the peripheral and central administration of PYY on food intake (40, 41). Peripheral injections of PYY caused significantly lower food intake in humans and rodents (including in a DIO mouse model) (2, 6, 11). Nevertheless, an intracerebroventricular injection of PYY induced higher food intake (42, 43). On the contrary, when PYY was injected directly into the hypothalamic arcuate nucleus, food intake was significantly decreased (6), an effect that was similar to that observed after a peripheral injection of PYY.

As for the difference in the effects of peripheral *vs.* central injections of PYY, one possible explanation could be that when PYY was injected peripherally, it was transported in the blood directly into areas in the brain with a high binding affinity to PYY, such as the hypothalamic arcuate nucleus (and possibly also the DVC), via the highly permeable blood-brain barrier. In these areas, PYY may have bound to the anorexigenic NPY Y2 receptor causing a decrease in food intake, which has been evidenced in Y2 knockout mice in which the anorectic effect of peripheral PYY injection was diminished (6). However, when injected centrally into the ventricle, it was likely that PYY bound to the more orexigenic NPY receptors, such as Y1 and Y5, caused an increase in food intake. This was also supported by the finding that the orexigenic effect of intracerebroventricular PYY was reduced in Y1 and Y5 receptor knockout mice (43).

In conclusion, it is clear that in diet-induced obese mice, there is a dysfunctional PYY regulation. Although there was an up-regulation in PYY and Y2 receptor binding in the medulla, the reduced amount of plasma PYY in the obese mice may have contributed to their high energy intake and subsequent weight gain.

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