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Social Isolation Affects the Development of Obesity and Type 2 Diabetes in Mice

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Social isolation is associated with increased risks of mortality and morbidity. In this study, we show that chronic individual housing accelerated body weight gain and adiposity in KK mice but not C57BL6J mice, and fully developed diabetes in KKA^y mice. Individually housed KK and KKA^y mice increased body weight gain over the initial 2 wk without increased daily average food consumption compared with group-housed animals. The individually housed KK and KKA^y mice then gradually increased food consumption for the next 1 wk. The chronic social isolation-induced obesity (SIO) was associated with hyperleptinemia and lower plasma corticosterone and active ghrelin levels but not hyperinsulinemia. Elevated plasma leptin in the SIO suppressed expression of 5-HT2C receptor in white adipose tissue. The SIO was also associated with decreased expression of β3-adrenergic receptors in white adipose tissue and hypothalamic leptin receptor, which might be secondary to the enhanced adiposity. Interestingly, social isolation acutely reduced food consumption and body weight gain compared with group-housed obese db/db mice with leptin receptor deficiency. Social isolation-induced hyperglycemia in KKA^y mice was associated with increased expression of hepatic gluconeogenetic genes independent of insulin. These findings suggest that social isolation promotes obesity due to primary decreased energy expenditure and secondary increased food consumption, which are independent of the disturbed leptin signaling, in KK mice, and develops into insulin-independent diabetes associated with increased expression of hepatic gluconeogenetic genes in KKA^y mice. Thus, social isolation can be included in the environmental factors that contribute to the development of obesity and type 2 diabetes. (*Endocrinology* 148: 4658–4666, 2007)

SOCIAL ISOLATION OR lack of social support is associated with increased risks for mortality (1, 2) and negative health outcomes, including heart disease, hypertension, stroke, and arthritis (3–7). Excess body weight during midlife is also associated with an increased risk factor of mortality and the negative health outcomes (8). However, interesting aspects of the relationship between social isolation and development of obesity remain to be resolved. Although individually housed Swiss CD-1 mice and Wistar rats do not grow as fast as group-housed ones (9–11), animal models of social isolation-induced obesity (SIO) and type 2 diabetes have yet to be identified.

The KK mice have long been included in the group of animals that become obese and develop diabetes, as have the A^y mice. The A^y yellow mice are known to become obese, and when bred with the KK mice, the development of diabetes is more pronounced. To determine the effects of social isolation on the development of obesity and type 2 diabetes, we examined the effects of individual and group housing on body weight, adipose tissues, plasma hormone levels, and expression of genes involved in the regulation of energy homeostasis in C57BL6J, KK, and KKA^y mice.

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Abbreviations: β3-AR, β3-Adrenergic receptor; BAT, brown adipose tissue; CNS, central nervous system; Fbp, fructose bisphosphatase; G6Pase, glucose-6-phosphatase; MC, melanocortin; PEPCK, pyruvate carboxykinase; SIO, social isolation-induced obesity; SOCS-3, suppressor of cytokine signaling 3; UCP, uncoupling protein.

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Materials and Methods

General procedures

Four-week-old male C57BL6J mice, KK, and KKA^y mice and 8-wk-old db/db mice were purchased from Japan CLEA (Tokyo, Japan). After the arrival of the animals, all mice were group-housed and acclimated to the colony for 1 wk before the experiment. Before the experiment, they were all housed (three to four mice per cage) with free access to water and chow pellets in a light (12 h on/12 h off; lights off at 2000 h)- and temperature (20–22 C)-controlled environment. One week later, animals were randomly transferred to individually housed conditions. Mice were housed in groups of three to four per cage (21.5 × 32 × 14 cm) or individually for 3 wk preceding decapitation. Their body weights were measured every 7 d in the morning for 3 wk. The average amounts of daily food consumption per week were evaluated in 5- to 8-wk-old animals.

Administration of drugs

Four-week-old C57BL6J mice were individually housed in standard mouse cages with free access to food and water for 1 wk before testing. Mice were injected ip with saline or leptin (5 mg/kg). They were not fed chow pellets. Sixty minutes later, the animals were decapitated and the epididymal white adipose tissue was removed for RNA extraction. The experiment was performed between 1000–1200 h. The dose of leptin (5 mg/kg) was selected based on the evidence that leptin induced hypophagia and decreases in body weights in 5-HT2C receptor mutant and wild-type mice (12).

Blood chemistries

Plasma ghrelin and adiponectin levels were measured by ELISA using an active ghrelin ELISA kit and des-acyl ghrelin ELISA kit (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan) and mouse adiponectin ELISA kit (Ootsuka Inc., Tokyo, Japan). For the ELISA of active ghrelin, 1 N hydrogen chloride was added to the samples at a final concentration of 0.1 N immediately after plasma separation. Plasma leptin, insulin, and corticosterone levels were measured using mouse leptin (Linco, St. Louis, MO), rat insulin (Linco), and rat corticosterone (ICN Biomedicals,

Costa Mesa, CA) RIA kits, respectively. Blood glucose levels were measured using glucose strips (blood glucose monitoring system; FreeStyle; KISSEI, Tokyo, Japan).

The animal studies were conducted under protocols in accordance with the institutional guidelines for animal experiments at Tohoku University Graduate School of Medicine.

Real-time quantitative RT-PCR

Total RNA was extracted from the epididymal white adipose tissue, brown adipose tissue (BAT), and soleus skeletal muscle by the acid-isolated guanidium thiocyanate-phenol-chloroform method and was isolated from mouse liver and hypothalamic tissue using the RNeasy Midi kit (Qiagen, Hilden, Germany) according to the manufacturer's directions. cDNA synthesis was performed using a Super Script III First-Strand Synthesis System for RT-PCR Kit (Invitrogen, Rockville, MD) using 1 μg total RNA. cDNA synthesized from total RNA was evaluated in a real-time PCR quantitative system (Light Cycler Quick System 350S; Roche Diagnostics, Mannheim, Germany). The primers used are listed in Table 1.

The relative amount of mRNA was calculated using β-actin mRNA as the invariant control. The data are shown as the fold change of the mean value of the control group, which received saline.

Data are presented as the mean values ± SEM (n = 5–8). Comparisons between the two groups were performed using two-tailed unpaired Student's *t* test. Comparisons among more than two groups were done by ANOVA using Bonferroni's test. The presence of a linear correlation was assessed using a parametric (Pearson's) correlation test. A *P* value of less than 0.05 was considered statistically significant.

TABLE 1. The primers used for real-time RT-PCR

Gene	Primer	Sequence
LepR	Sense	CTGAATTTCCAAAAGCCTGA
	Antisense	AAGCTGTATCGACACTGATTTC
MC4R	Sense	GAGGTGTTTGTGACTCTGGG
	Antisense	GAACATGTGGACATAGAGAG
5-HT2CR	Sense	CTGAGGGACGAAAGCAAAG
	Antisense	CACATAGCCAATCCAAACAAAC
5-HT1BR	Sense	TGCCTGCTGGTTTCACAT
	Antisense	GCGCACTTAAAGCGTATCA
SOCS-3	Sense	GCGGGCACCTTTCTTATCC
	Antisense	TCCCCGACTGGGTCTTGAC
β3-AR	Sense	ATGGCTCCGTGGCCTCAC
	Antisense	CCCAACGGCCAGTGGCCAGTCAGCG
UCP-1	Sense	GACAGTACCCAAGCGTACCAA
	Antisense	CATGATGACGTTCCAGGACC
UCP-2	Sense	GTTCCTCTGTCTCGTCTTGC
	Antisense	GGCCTTGAAACCAACCA
UCP-3	Sense	GTTGCTGGAGTCTCACCTGT
	Antisense	TCTTCAGCATACAGTGCAGA
PPARα	Sense	CGGGTAACCTCGAAGTCTGA
	Antisense	CTAACCTTGGGCCACACCT
PPARγ	Sense	CTGCTCAAGTATGGTGTCCATGAG
	Antisense	GAGGAACTCCCTGGTCATGAATC
PPARδ	Sense	GCTGCTGCAGAAGATGGCA
	Antisense	CACTGCATCATCTGGGCATG
G6Pase	Sense	TGCAAGGGAGAACTCAGCAA
	Antisense	GGACCAAGGAAGCCACAATG
Fbp1	Sense	TCTGCACCGCGATCAAAG
	Antisense	GTTGAGCCAGCGATACCATAGAG
Fbp2	Sense	AGAAAGACCACGGAGGACGA
	Antisense	CCCGCAGCCACGATGT
PEPCK	Sense	AGCGGATATGGTGGGAAC
	Antisense	GGTCTCCACTCCTTGTTC
β-actin	Sense	TTGTAACCAACTGGGACGATATGG
	Antisense	GATCTTGATCTTCATGGTGCTAGG

Results

Changes in body weight and adipose tissue weight in individually and group-housed C57BL6J mice and KK mice

There were no significant differences in body weight change between individually housed and group-housed C57BL6J mice for 3 wk in 6- to 8-wk-old animals (Fig. 1A), whereas the increases in body weight were significantly greater in individually housed KK mice than those in group-housed ones after 6 wk of age (Fig. 1B). Epididymal white adipose tissue and BAT weight significantly increased in 8-wk-old individually housed KK mice compared with group-housed ones, whereas there were no differences between individually housed and group-housed C57BL6J mice (Fig. 1, C and D). These findings indicate that chronic social isolation accelerates body weight gain and adiposity in KK mice but not C57BL6J mice.

Plasma chemistries in individually housed and group-housed KK mice

To determine the characteristics of obesity induced by chronic social isolation, we examined blood chemistries in individually housed and group-housed 8-wk-old C57BL6J and KK mice. Plasma leptin levels were significantly elevated in individually housed 8-wk-old KK mice compared with group-housed ones (15.5 ± 1.1 *vs.* 6.01 ± 0.55 ng/ml, $P < 0.05$). There were no significant differences in plasma insulin levels between the individually housed and group-housed 8-wk-old KK mice (5.34 ± 0.94 *vs.* 3.90 ± 0.62 ng/ml). Plasma corticosterone and active ghrelin, but not des-acyl ghrelin, levels were significantly decreased in the individually housed 8-wk-old KK mice compared with group-housed ones (corticosterone, 23.9 ± 2.7 *vs.* 56.3 ± 11.9 ng/ml, $P < 0.05$; active ghrelin, 8.72 ± 0.94 *vs.* 14.88 ± 1.37 fmol/ml, $P < 0.05$; and des-acyl ghrelin, 258 ± 21.0 *vs.* 288 ± 30.17 fmol/ml). There were no significant differences in the plasma adiponectin or blood glucose levels between the individually housed and group-housed 8-wk-old KK mice (adiponectin, 7.12 ± 0.29 *vs.* 7.62 ± 0.59 μg/ml; glucose, 156 ± 9 *vs.* 160 ± 5 mg/dl). These hormonal and metabolic alterations induced by social isolation in KK mice were not found in C57BL6J mice (data not shown). These findings suggest that chronic SIO is not due to hyperinsulinemia or hypercorticosteronemia, and is not associated with decreases in plasma adiponectin or des-acyl ghrelin levels.

Altered expression of genes involved in energy homeostasis and daily food consumption of individually housed and group-housed KK mice

To further determine the characteristics of chronic SIO associated with hyperleptinemia in the individually housed and group-housed 8-wk-old KK mice (Fig. 2A), we examined the expression of hypothalamic leptin receptor (Ob-Rb; *LepR*), melanocortin (MC)-4 receptor, and 5-HT2C receptor, which are involved in the central regulation of feeding behavior and energy homeostasis (13). Hypothalamic *LepR* mRNA levels were significantly decreased in the individually housed 8-wk-old KK mice compared with group-housed mice (24% decrease), although there were no significant dif-

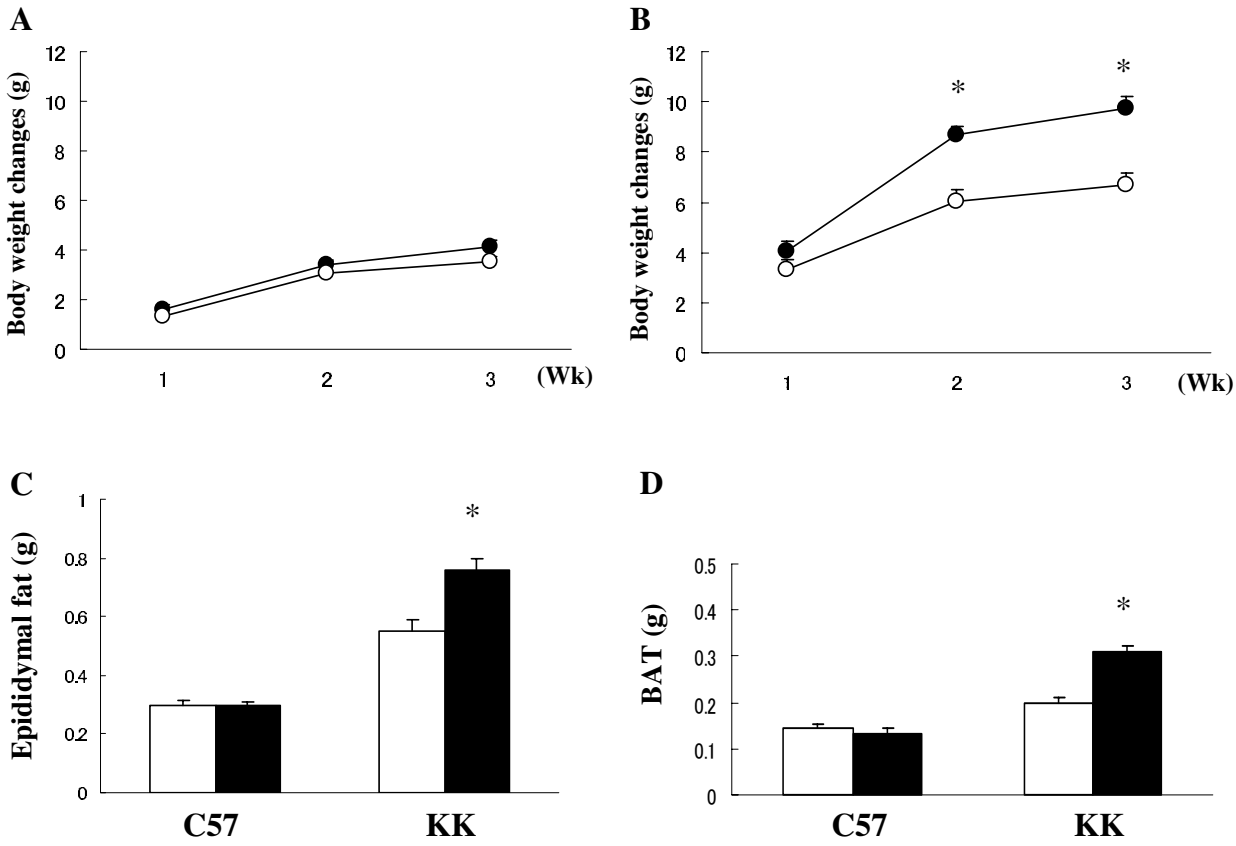

FIG. 1. Body weight changes for 3 wk from the period of 5 to 8 wk old in individually housed (*solid circles*) and group-housed (*open circles*) C57BL6J mice (A) and KK mice (B). Epididymal white adipose tissue (C) and BAT weight (D) in individually housed (*filled bars*) and group-housed (*open bars*) 8-wk-old C57BL6J mice and KK mice as described in *Materials and Methods*. Basal body weight: individually housed and group-housed C57BL6J mice were 18.5 ± 0.2 and 18.3 ± 0.2 g, respectively; individually housed and group-housed KK mice were 22.0 ± 0.2 and 22.0 ± 0.2 g, respectively. Data are presented as the mean values ± SEM (n = 8). C57, C57BL6J mice; KK, KK mice. *, $P < 0.05$.

ferences in hypothalamic MC-4 receptor or 5-HT2C receptor mRNA levels (Fig. 2A). In addition, there were no significant differences in mRNA levels of hypothalamic suppressor of cytokine signaling (SOCS)-3, which is related to the central leptin resistance (14–16), between the two groups (Fig. 2A).

Leptin increases central sympathetic outflow to white adipose tissue via β3-adrenergic receptor (β3-AR), leading to increased lipolysis (13, 17). Mice with a null mutation of the β3-AR gene have a mild increase in fat stores at an early age (18). A disturbance of sympathetic neural action on adipose tissues by β3-AR results in increased fat stores without hyperphagia (13, 17, 18). The present study demonstrates that the mRNA levels of β3-AR in epididymal white adipose tissue were significantly decreased in the 8-wk-old individually housed KK mice compared with the group-housed ones (Fig. 2B).

The expression of 5-HT2C receptors appears to be restricted to the central nervous system (CNS) (19). Interestingly, the present study demonstrates that the 5-HT2C receptor is expressed in epididymal white adipose tissue, and the mRNA levels of the 5-HT2C receptor but not the 5-HT1B receptor in epididymal white adipose tissue were significantly decreased in 8-wk-old individually housed KK mice compared with the group-housed animals (Fig. 2C). Plasma leptin levels and the 5-HT2C receptor mRNA levels in white adipose tissue were inversely correlated ($r = -0.84$, $P = 0.0012$) (Fig. 2D).

There were no significant differences in average daily food consumption between the two groups for the initial 2 wk, and then individually housed KK mice significantly increased food consumption compared with the group-housed KK mice for the next 1 wk (Fig. 2E).

Effects of leptin on expression of 5-HT2C receptor in white adipose tissue

To further determine the effects of leptin on 5-HT2C receptor expression in white adipose tissue, we examined exogenous administration of leptin on 5-HT2C receptor mRNA levels in epididymal white adipose tissue in C57BL6J mice. Intraperitoneally administration of leptin (5 mg/kg) dramatically decreased 5-HT2C receptor but not 5-HT1B receptor mRNA levels in epididymal white adipose tissue compared with saline controls (Fig. 2F). These findings suggest that leptin down-regulates the expression of 5-HT2C receptor in white adipose tissue independent of feeding behavior.

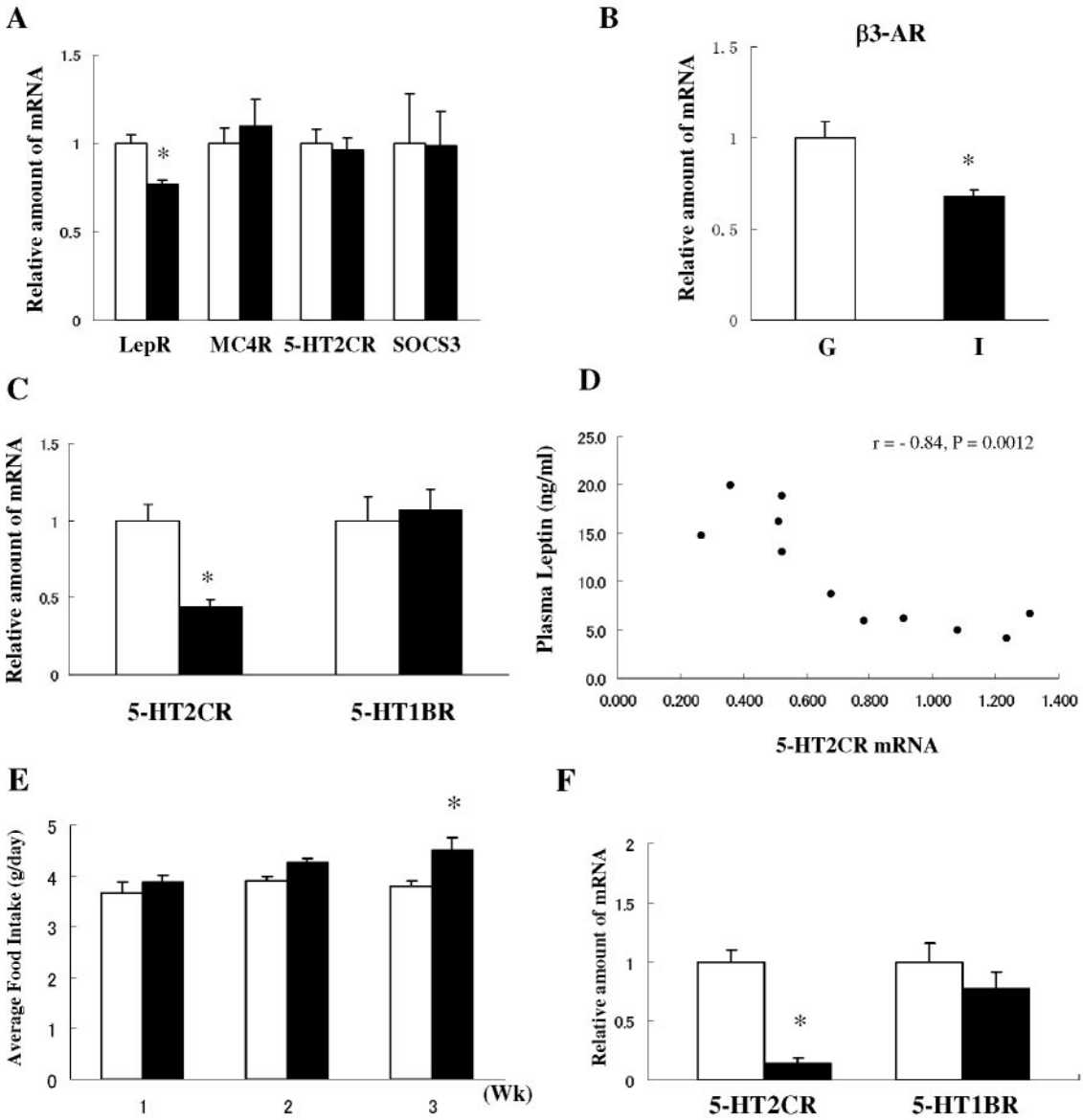

FIG. 2. Expression of *LepR*, *MC4R*, *5-HT2CR*, and *SOCS-3* genes in the hypothalamus (A), and expression of *β3*-AR gene (B) and *5-HT2C* receptor gene (C) in the epididymal white adipose tissue, and the relationship between plasma leptin levels and *5-HT2C* receptor gene expression in epididymal white adipose tissue (D), and average daily food consumption per week of 8-wk-old individually housed (*filled bars*) and group-housed (*open bars*) KK mice (E) as described in *Materials and Methods*. Effects of leptin on 5-HT2C receptor and 5-HT1B receptor mRNA levels in the epididymal white adipose tissue (F) of 5-wk-old C57BL6J mice, as described in *Materials and Methods*. Data are presented as the mean values ± SEM (n = 5–6). LepR, Leptin receptor; MC4R, MC-4 receptor, 5-HT2CR, serotonin 5-HT2C receptor; I, individually housed animals; G, group-housed animals. *, $P < 0.05$.

Altered expression of uncoupling proteins (UCPs) in adipose tissues and skeletal muscle of individually housed and group-housed KK mice

UCPs on the mitochondrial inner membrane are effectors for adaptive thermogenesis (20). The expression of UCP-1 in BAT and the expression of UCP-2 in white adipose tissue has been suggested to increase in response to high-fat diet (21). The present study demonstrates that UCP-2 mRNA levels in epididymal white adipose tissue were significantly decreased in individually housed 8-wk-old KK mice compared with group-housed mice, and there were no significant differences in UCP-1 mRNA levels in BAT, UCP-2 mRNA levels in the liver or the soleus muscle between the 8-wk-old individually housed and group-housed KK mice (Table 2).

Altered expression of PPARs in adipose tissue, skeletal muscle and liver of individually housed and group-housed KK mice

The nuclear receptor peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that function as fatty acid-activated transcription factors (22). Among three related PPAR family members: PPARα, PPARγ, and PPARδ, the expression of PPARγ and PPARα in epididymal white adipose tissue and skeletal mus-

TABLE 2. Altered UCP-1 mRNA levels in the BAT, UCP-2 mRNA levels in the epididymal white adipose tissue (WAT) and soleus muscle, and UCP-3 mRNA levels in the soleus muscle of individually housed and group-housed 8-wk-old KK mice

		G	I
UCPs			
BAT	UCP-1	1 ± 0.08	1.10 ± 0.05
WAT	UCP-2	1 ± 0.10	0.61 ± 0.21[a]
Muscle	UCP-2	1 ± 0.12	0.93 ± 0.14
	UCP-3	1 ± 0.20	1.10 ± 0.21
PPARs			
WAT	PPARδ	1 ± 0.06	0.80 ± 0.07
	PPARα	1 ± 0.10	1.20 ± 0.10
	PPARγ	1 ± 0.12	1.20 ± 0.12
Muscle	PPARδ	1 ± 0.06	1.00 ± 0.16
	PPARα	1 ± 0.06	0.90 ± 0.08
	PPARγ	1 ± 0.10	0.66 ± 0.05[a]

Altered PPARδ, PPARα, and PPARγ mRNA levels in the WAT and soleus muscle of individually and group housed 8-wk-old KK mice, as described in *Materials and Methods*. Data are presented as the mean values ± SEM (n = 6). I, Individually housed KK mice; G, group housed KK mice.

[a] $P < 0.05$.

cle is reportedly increased in response to high-fat diet (23–25). The present study demonstrates that there were no differences in either PPARγ or PPARα mRNA levels in the epididymal white adipose tissue or the soleus muscle between individually housed and group-housed 8-wk-old KK mice (Table 2).

PPARδ enhances fatty acid catabolism and energy uncoupling in white adipose tissue and/or skeletal muscle, leading to prevention of diet-induced obesity (26, 27). However, the present results demonstrate that there were no significant differences in PPARδ mRNA levels in the epididymal white adipose tissue or the soleus muscle between individually housed and group-housed 8-wk-old KK mice (Table 2).

The increased expression of PPARγ and PPARα in the liver is a common characteristic of obese rodents including ob/ob mice, db/db mice, and obese 5-HT2C receptor mutant mice (28). The present study also demonstrates that PPARγ and PPARα mRNA levels in the liver were significantly increased in 8-wk-old individually housed KK mice compared with group-housed ones (Fig. 3, A and B). There were no significant differences in expression of gluconeogenetic genes such as glucose-6-phosphatase (G6Pase), fructose bisphosphatase (Fbp) 1, and Fbp2 in the liver of 8-wk-old individually housed and group-housed KK mice (Fig. 3C). These findings support the finding that there were no differences in blood glucose levels between the individually housed and group-housed KK mice. Altered expression of these genes was not found between the 8-wk-old individually housed and group-housed C57BL6J mice (data not shown).

Development of diabetes in individually housed and group-housed KKA^y mice

A^y mice have dominant alleles at the agouti locus (*A*), which produces ectopic expression of the agouti peptide, an antagonist of the hypothalamic MC-4 receptors and MC-3

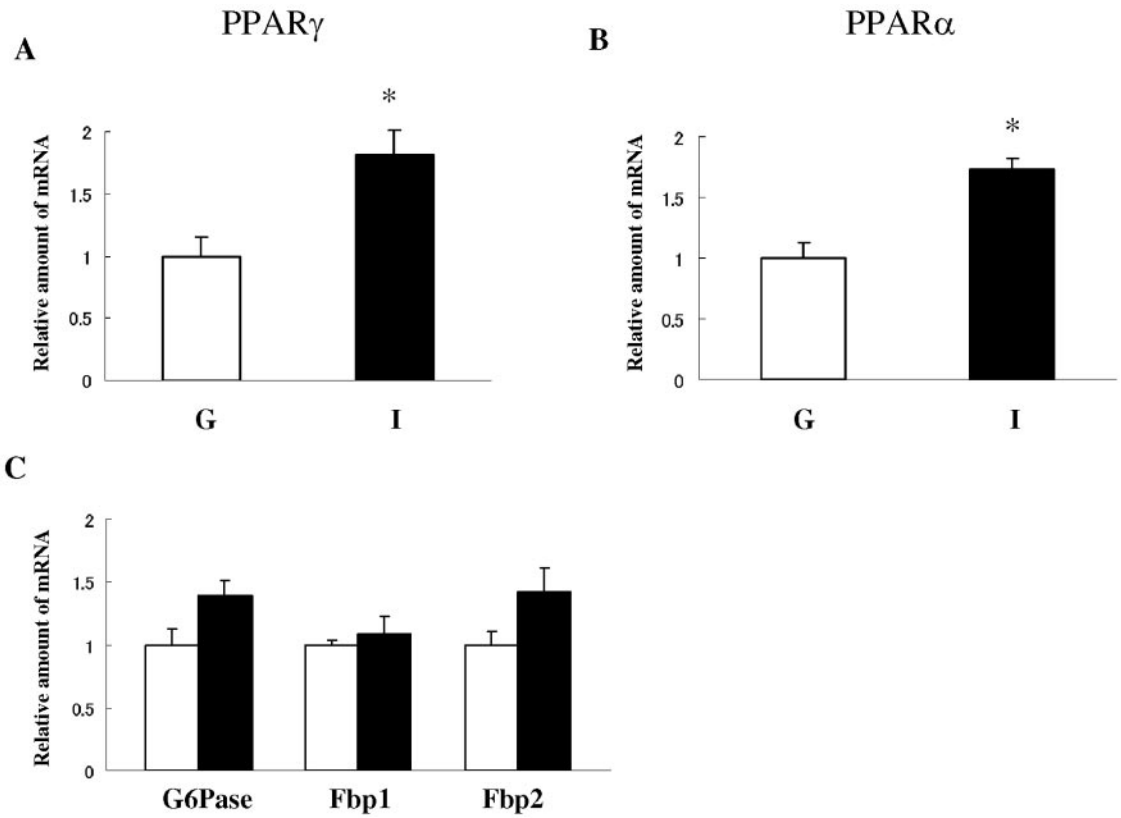

FIG. 3. Altered PPARγ (A), PPARα (B), and GP6ase, Fbp1, and Fbp2 (C) mRNA levels in the liver of 8-wk-old individually housed (*filled bars*) and group-housed (*open bars*) KK mice, as described in *Materials and Methods*. Data are presented as the mean values ± SEM (n = 5–6). I, Individually housed animals; G, group-housed animals. *, $P < 0.05$.

receptors, and display hyperphagia, obesity, and diabetes (29–31). Individually housed 8-wk-old KKA^y mice displayed hyperglycemia in association with increased body weight, epididymal white adipose tissue weight, and plasma leptin levels compared with group-housed KKA^y mice (Fig. 4, A–E), whereas there were no significant differences in plasma insulin or adiponectin levels between individually housed and group-housed KKA^y mice (Fig. 4, F and I). The plasma active ghrelin levels were remarkably decreased (Fig. 4G), and des-acyl ghrelin levels were slightly decreased in

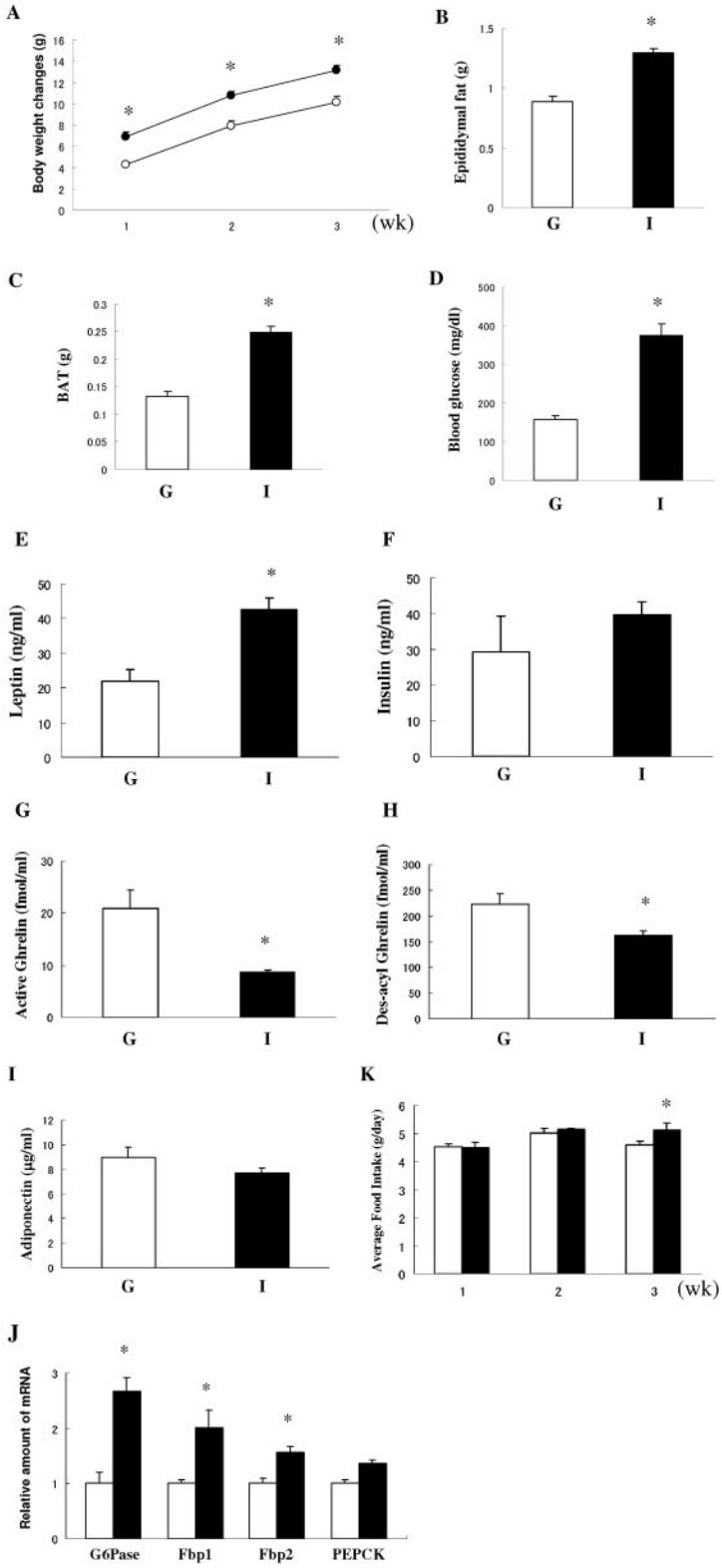

FIG. 4. Body weight changes for 3 wk in the period from 5- to 8-wk-old individually housed (*solid circles*) and group-housed (*open circles*) KKA^y mice (A). Epididymal white adipose tissue (B) and BAT weight (C), blood glucose (D), plasma leptin (E), insulin (F), active ghrelin (G), des-acyl ghrelin (H), and adiponectin levels (I), and GP6ase, Fbp1, Fbp2, and PEPCK mRNA levels (J) in the liver of 8-wk-old individually housed (*filled bars*) and group-housed (*open bars*) KKA^y mice as described in *Materials and Methods*. Average daily food consumption for the week that transpired (K) in the period from 5- to 8-wk-old individually housed (*solid circles* and *filled bars*) and group-housed (*open circles* and *open bars*) KKA^y mice. Basal body weight: individually housed and group-housed KKA^y mice 24.5 ± 0.2 and 24.5 ± 0.2 g, respectively. Data are presented as the mean values ± SEM (n = 6–8). I, Individually housed animals; G, group-housed animals. *, $P < 0.05$.

individually housed KKA^y mice compared with group-housed KKA^y mice (Fig. 4H). Individually housed 8-wk-old KKA^y mice exhibited increased expression of the hepatic G6Pase, Fbp1, and Fbp2 genes, which are involved in gluconeogenesis (32), whereas there were no significant effects on hepatic pyruvate carboxykinase (PEPCK) mRNA levels (Fig. 4J). These findings suggest that chronic social isolation can fully develop into insulin-independent diabetes associated with increased hepatic gluconeogenetic genes in addition to obesity in KKA^y mice. There were no significant differences in average daily food consumption between the two groups for the initial 2 wk, and then the individually housed KKA^y mice slightly increased food consumption compared with the group-housed animals for the next 1 wk (Fig. 4K).

Effects of social isolation on food consumption and body weight gain in db/db mice

To further determine the physiological role of the decreased expression of the hypothalamic *LepR* gene, we examined body weight gain and daily food consumption in individually housed and group-housed obese db/db mice. Body weight gain was significantly lower in the individually housed than group-housed db/db mice after 9 wk of age (Fig. 5A). In addition, daily food consumption was relatively lower in individually housed db/db mice than the group-housed animals (Fig. 5B), and the average amount of daily food consumption per week in the 9- to 10-wk-old animals was significantly lower in individually housed than group-housed db/db mice (Fig. 5C).

Discussion

The present study demonstrates that chronic individual housing accelerated body weight gain and adiposity in KK and KKA^y mice but not C57BL6J mice. First, the social isolation-induced body weight gain in the KK strains occurred without increased food consumption, suggesting that decreased energy expenditure primarily contributes to the accelerated body weight gain. Subsequently, the SIO developed in association with slightly increased food consumption.

The SIO displays certain characteristics distinct from the general features of diet-induced obesity. The first reason in support of this is based on the result that despite lower active ghrelin, there were no differences in plasma des-acyl ghrelin levels between the individually housed and group-housed KK mice. We previously reported that hyperphagia decreases plasma des-acyl ghrelin, but not active ghrelin, levels in mice (31). The second reason is based on the result that hepatic UCP-2 gene expression was not increased in the individually housed KK mice. UCP-2 gene expression in the liver is increased in hyperphagic 5-HT2C receptor mutant mice (33). The third reason is based on the result that UCP-1 expression in BAT and UCP-2 expression in white adipose tissue were not increased in the individually housed KK mice. The UCP-1 expression in BAT and UCP-2 expression

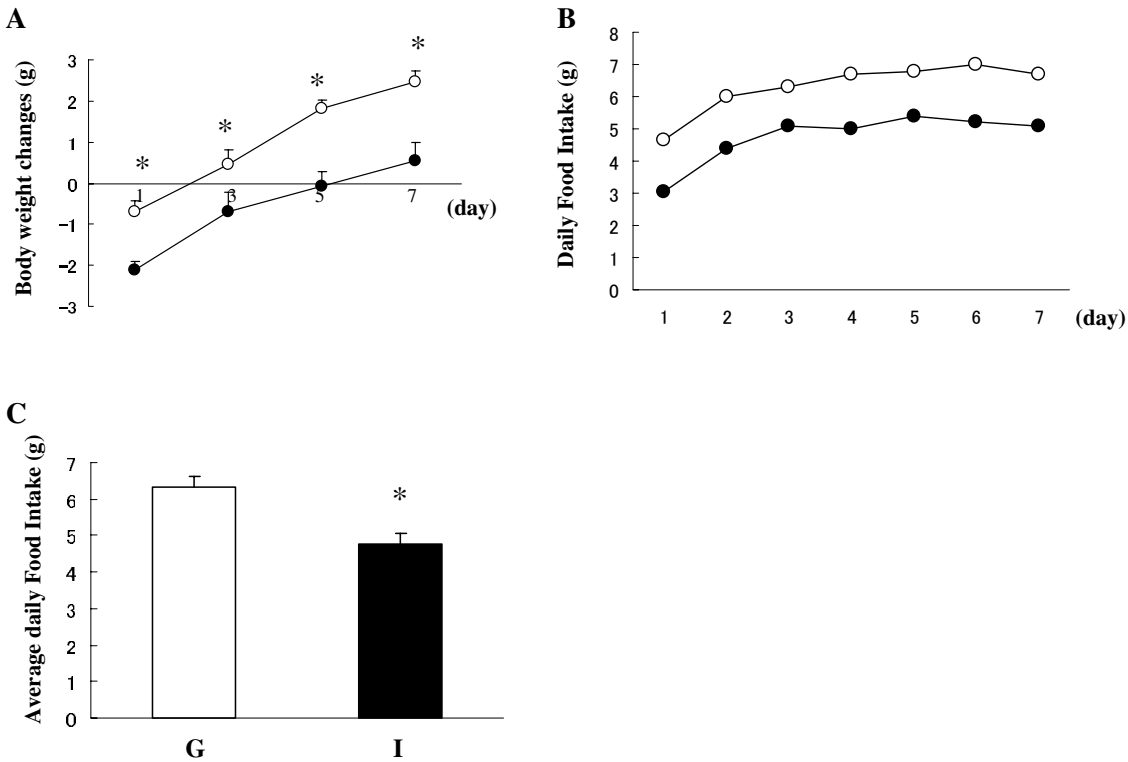

FIG. 5. Body weight changes (A), daily food consumption (B), and average daily food consumption for the week that transpired (C) in the period from 9- to 10-wk-old individually housed (*solid circles* and *filled bars*) and group-housed (*open circles* and *bars*) db/db mice. Basal body weights of the individually housed and group-housed db/db mice were 41.3 ± 0.3 and 41.5 ± 0.4 g, respectively. Data are presented as the mean values ± SEM (n = 7). I, Individually housed animals; G, group-housed animals. *, $P < 0.05$.

in white adipose tissue are increased in responses to high-fat diet (21). The fourth reason is based on the result that the expression of PPARs in white adipose tissue and soleus muscle was not increased in the individually housed KK mice. The expression of PPARα and PPARγ in white adipose tissue and skeletal muscle is increased in responses to high-fat diet (23–25). The fifth reason is based on the result of hypothalamic SOCS-3 gene expression, because hypothalamic SOCS-3 has an inhibitory role in diet-induced obesity (14–16). Thus, it is possible that decreased energy expenditure rather than increased energy intake might primarily contribute to the social isolation-induced adiposity.

Circulating leptin signals the CNS, first to rapidly increase sympathetic outflow and then to inhibit food intake (13, 17). The sympathetic nervous system increases lipolysis and suppresses leptin expression in white adipose tissue through the β3-AR (13, 17). Thus, there is a negative feedback system between sympathetic nervous system stimulation and leptin production. Therefore, dysfunction of autonomic neural circuits between white adipose tissue and the CNS contributes to the development of obesity (13, 17). However, our present results demonstrate that social isolation decreased body weight gain in association with decreased daily food consumption in obese db/db mice, suggesting that disturbed leptin signaling does not contribute to the causes of SIO. Therefore, the decreased expression of hypothalamic *Lep*R might be a secondary response to the enhanced adiposity induced by chronic social isolation. These findings suggest central neural mechanisms independent of leptin signaling contribute to the development of the SIO.

The central serotonin and leptin signaling contribute substantially to the regulation of feeding and energy homeostasis. The expression of 5-HT2C receptors appears to be restricted to the CNS (19). Mice with a null mutation of the 5-HT2C receptor gene elevate body weight, and are resistant to the anorexic effects of meta-chlorophenylpiperazine, indicating that 5-HT2C receptors contribute substantially to the serotonin regulation of body weight (19). Despite hyperphagia, 5-HT2C receptor mutant mice do not develop obesity until 6 months of age, because of increased physical activity (12, 34). Chronic hyperphagia and hyperactivity lead to a late onset obesity associated with hyperleptinemia in 5-HT2C receptor mutant mice because of decreased energy cost of physical activity (12, 34). 5-HT2C receptor has been suggested to regulate feeding behavior and physical activity rather than direct neural regulation of fat metabolism. Despite hyperactivity, pair-feeding, however, does not decrease body weight in 5-HT2C receptor mutants compared with wild-type mice (19). The present study demonstrates that white adipose tissue expresses serotonin 5-HT2C receptor, and the leptin-induced inhibition of 5-HT2C receptor expression in white adipose tissue might be an additive factor for the enhanced adiposity independent of feeding. The direct effects of 5-HT2C receptor gene on the white adipose tissue *in vivo* warrant further examination in the future.

Hepatic gluconeogenesis contributes to hyperglycemia in type 2 diabetes. Increased G6Pase, Fbp1, and Fbp2 genes involved in hepatic gluconeogenesis are associated with increased glucose production and blood glucose levels in db/db mice with insulin resistance and streptozocin-induced diabetic animals with insulin deficiency (32). Given our results, insulin-independent diabetes induced by chronic social isolation was also associated with increased expression of hepatic gluconeogenetic genes such as G6Pase, Fbp1, and Fbp2, but not PEPCK in KKA^y mice. PEPCK, a rate-limiting enzyme in the gluconeogenic pathway, is required for glucose synthesis from pyruvate, but is not required for glucose production from other carbon precursors such as glycerol. Given our results, an increased gluconeogenic pathway other than glucose synthesis from pyruvate might contribute to the chronic social isolation-induced hyperglycemia in the KKA^y mice. Hyperglycemia in individually housed KKA^y mice is due to hyperphagia, which decreases plasma des-acyl ghrelin levels (31). Agouti peptide, an endogenous MC-4 receptor antagonist, in addition to chronic social isolation, might induce hyperphagia, leading to the hyperglycemia in KKA^y mice.

In summary, these results suggest that social isolation promotes leptin-independent adiposity in KK mice and develops into insulin-independent diabetes associated with increased expression of hepatic gluconeogenetic genes in KKA^y mice. Thus, social isolation can be included in the environmental factors related to the development of obesity and type 2 diabetes, and group housing can apparently prevent or at least mitigate it.

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K.Non., K.Noz., and Y.O. have nothing to declare.

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