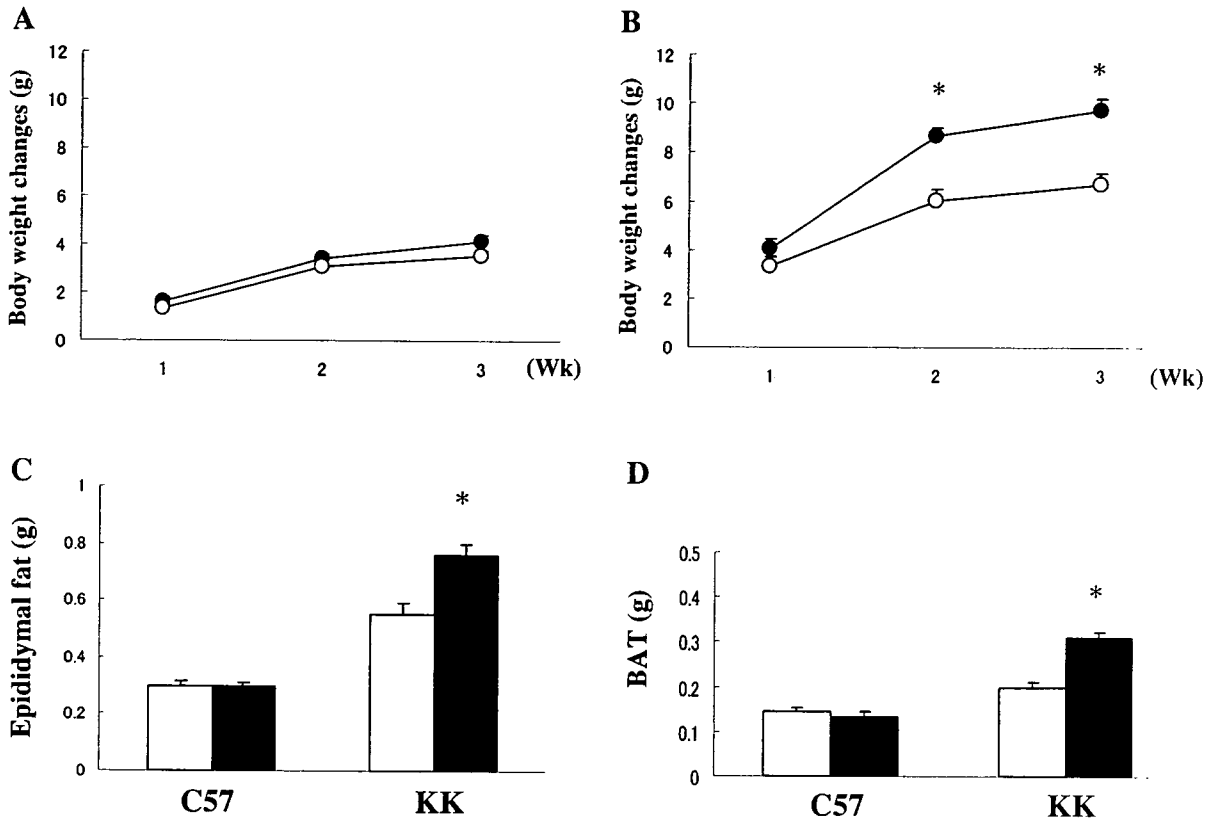


FIG. 1. Body weight changes for 3 wk from the period of 5 to 8 wk old in individually housed (*solid circles*) and group-housed (*open circles*) C57BL6J mice (A) and KK mice (B). Epididymal white adipose tissue (C) and BAT weight (D) in individually housed (*filled bars*) and group-housed (*open bars*) 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.

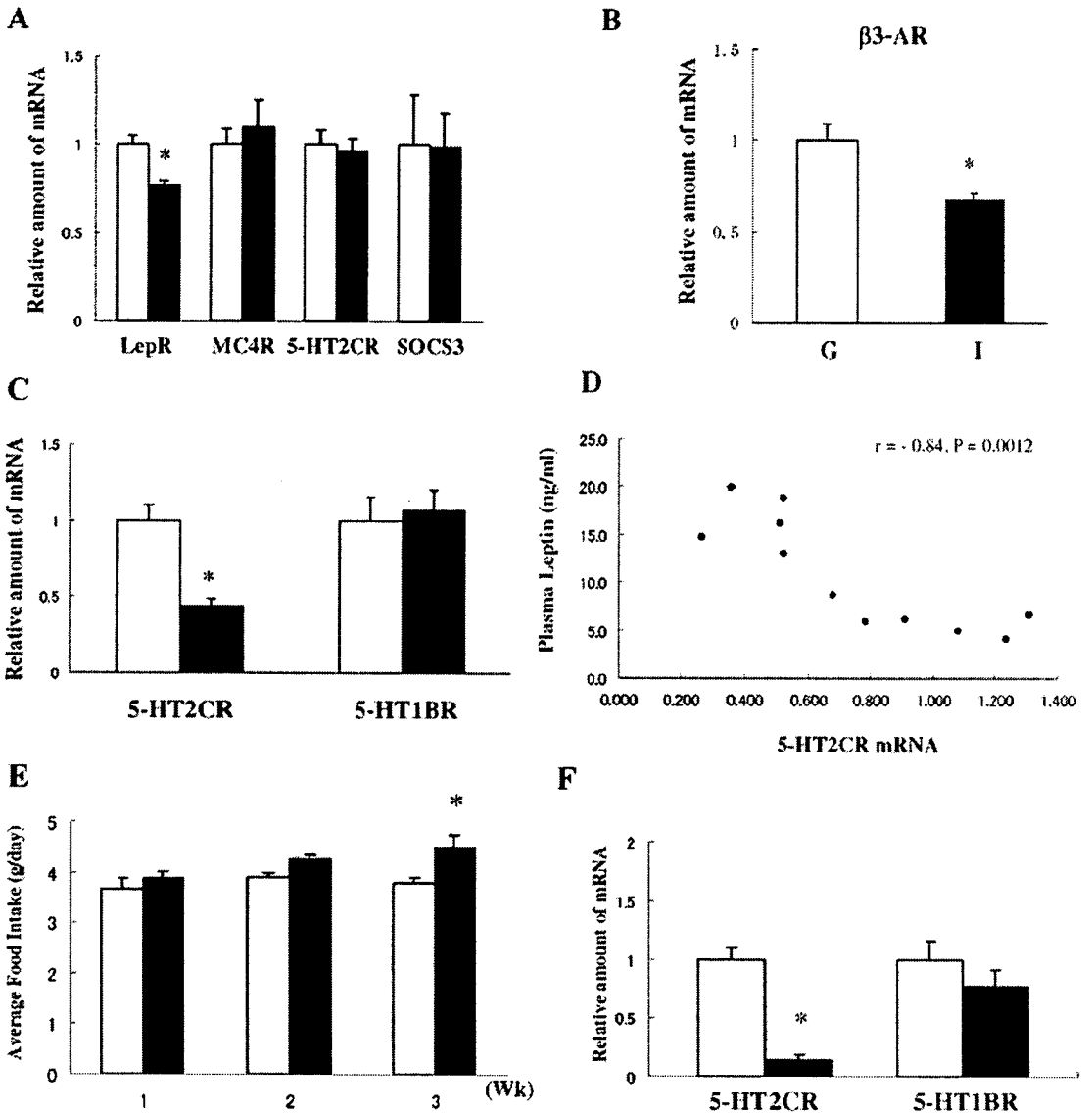


FIG. 2. Expression of *LepR*, *MC4R*, *5-HT2CR*, and *SOCS-3* genes in the hypothalamus (A), and expression of *β3*-AR gene (B) and *5-HT2C* receptor gene (C) in the epididymal white adipose tissue, and the relationship between plasma leptin levels and *5-HT2C* receptor gene expression in epididymal 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

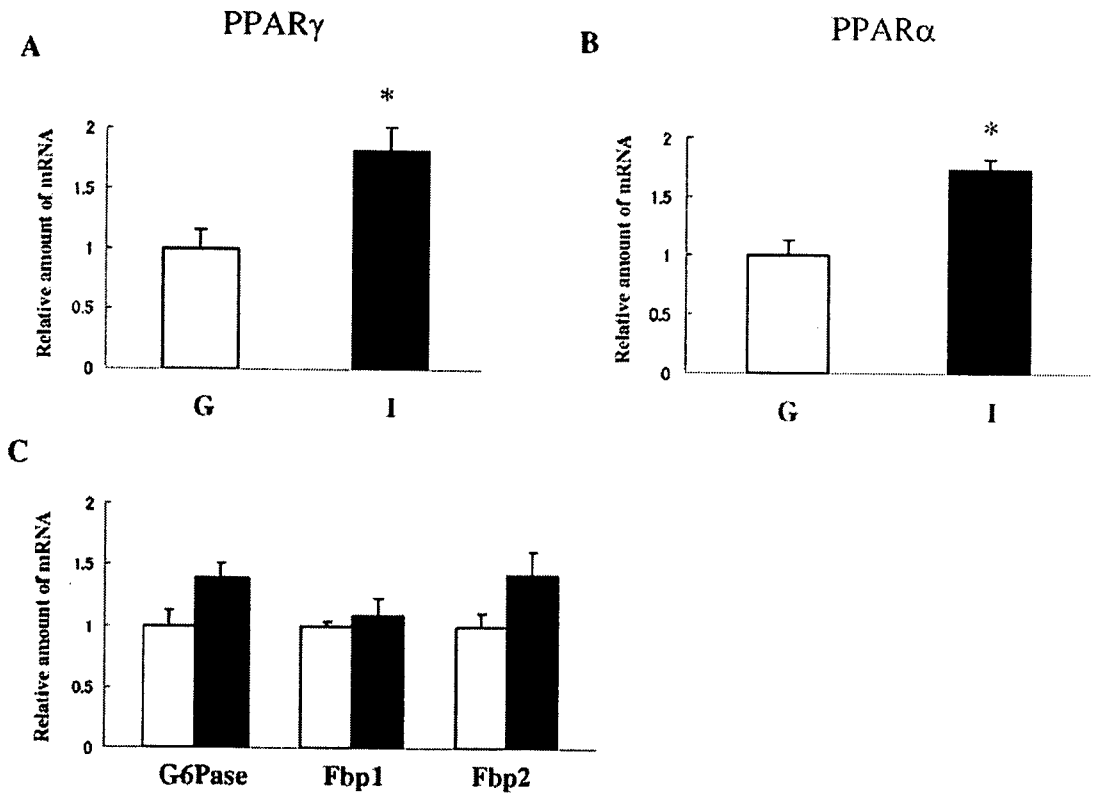


FIG. 3. Altered PPARγ (A), PPARα (B), and GP6ase, Fbp1, and Fbp2 (C) mRNA levels in the liver of 8-wk-old individually housed (*filled bars*) and group-housed (*open bars*) KK mice, as described in *Materials and Methods*. Data are presented as the mean values ± SEM (n = 5–6). I, Individually housed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

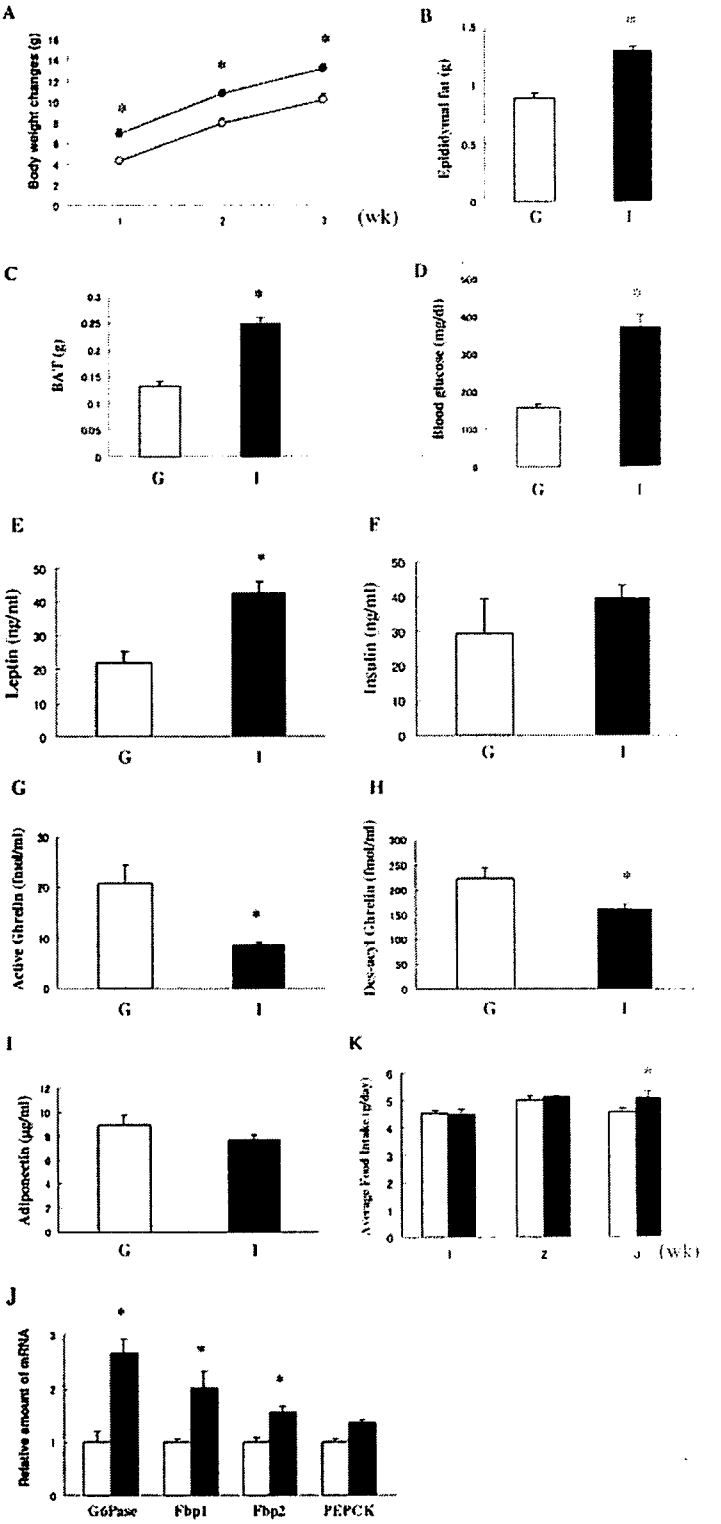


FIG. 4. Body weight changes for 3 wk in the period from 5- to 8-wk-old individually housed (*solid circles*) and group-housed (*open circles*) KKA$^y$ mice (A). Epididymal white adipose tissue (B) and BAT weight (C), blood glucose (D), plasma leptin (E), insulin (F), active ghrelin (G), des-acyl ghrelin (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

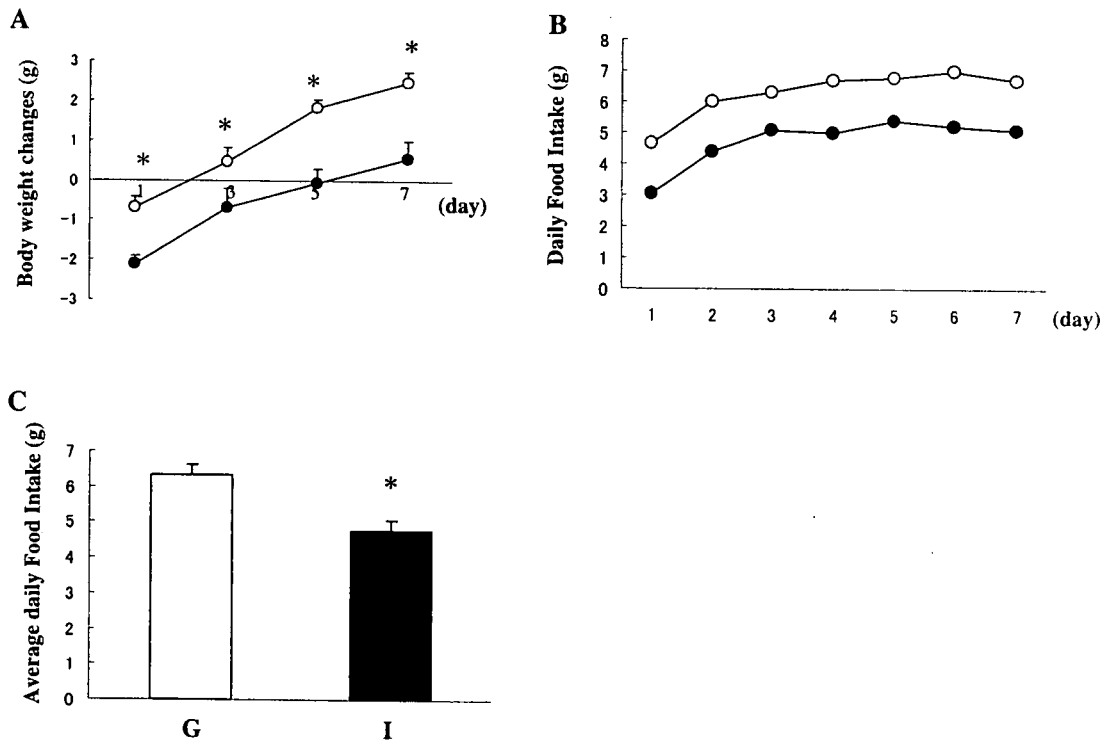


FIG. 5. Body weight changes (A), daily food consumption (B), and average daily food consumption for the week that transpired (C) in the period from 9- to 10-wk-old individually housed (*solid circles* and *filled bars*) and group-housed (*open circles* and *bars*) db/db mice. Basal body weights of the 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 PPAR$\alpha$ and PPAR$\gamma$ 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 $\beta$3-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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OBSERVATIONS

# Possible Relevance of HLA-DRB1*0403 Haplotype in Insulin Autoimmune Syndrome Induced by α-Lipoic Acid, Used as a Dietary Supplement

Insulin autoimmune syndrome (IAS) is characterized by frequent hypoglycemic attacks associated with the presence of autoantibodies to insulin in patients who have not received insulin injections. Approximately half of IAS patients have a medication history before onset, and over 90% of the agents are sulfydryl compounds such as methimazole, mercaptopropionyl glycine, or glutathione. In addition to these compounds, α-lipoic acid (ALA), which is widely used as a health supplement, is associated with a risk of IAS induction, as previously reported in *Diabetes Care* (1) and other journals (2,3). DRB1*0406 is reportedly the most common and DRB1*0403 the next most common HLA haplotype conferring susceptibility to IAS (4). As for ALA-induced IAS, all three reported cases have the DRB1*0406 but not the DRB1*0403 haplotype (1–3). However, we recently observed a case of IAS, possibly induced by ALA, in a patient who has the DRB1*0403 haplotype.

The patient, a 45-year-old woman, lapsed into hypoglycemic coma 1 month after starting to take ALA. She had not taken any of the other aforementioned sulfydryl compounds. She exhibited marked hyperinsulinemia (fasting plasma glucose 88 mg/dl, serum immunoreactive insulin 13,240 μU/ml, and serum C-peptide immunoreactivity 2.93 ng/ml). Antibodies to insulin were detected with an insulin binding ratio of 81.2%. Antibody affinity was low, while binding activity was high, as commonly observed in IAS. Based on these results, she was diagnosed as having IAS possibly induced by ALA. However, she has the DRB1*0403, not the DRB1*0406, haplotype.

This is the first report of a patient with ALA-induced IAS having the DRB1*0403 haplotype. Since the DRB1*0403 haplotype is reportedly associated with IAS induced by other sulfydryl compounds, it is likely to confer susceptibility to ALA-induced IAS. Although IAS was a relatively rare cause of hypoglycemia in the past, ALA has become more widely available as a dietary supplement for treating obesity and diabetes complications. Furthermore, in contrast to the very low prevalence of DRB1*0406 in ethnic groups other than East Asians, DRB1*0403 was found to be widely distributed across various populations (5). We should therefore be more aware of ALA-induced IAS.

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REVIEW

# Avenues of Communication between the Brain and Tissues/Organs Involved in Energy Homeostasis

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**Abstract.** Obesity is a rapidly increasing public health concern worldwide as a major risk factor for numerous disorders, including diabetes, hypertension and heart disease. Despite remarkable advances in obesity research over the past 10 years, the molecular mechanisms underlying obesity are still not completely understood. To maintain systemic energy homeostasis, it is important that organs/tissues communicate metabolic information among each other. Obesity-related disorders can be thought of as resulting from dysregulation of this inter-tissue communication. This system has both afferent sensing components and efferent effecter limbs. The afferent signals consist of not only humoral factors, such as nutrients (glucose, fatty acids and amino acids) and adipocytokines (leptin, adiponectin and so on), but also autonomic afferent nerve systems. Both converge on brain centers, most importantly within the hypothalamus, where the signals are integrated, and the direction and magnitude of efferent responses are determined. The efferent elements of this physiological system include those regulating energy inputs and outputs, i.e. food intake and metabolic rates. In this review, we will summarize recent advances in research on metabolic information avenues to the brain, which are important for energy homeostasis.

*Key words*:

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**THE** worldwide prevalence of obesity, which is a major risk factor for numerous disorders, including diabetes, hypertension and heart disease, is increasing at an alarming rate, with major adverse consequences for human health [1]. Body weight is thought to be determined by the balance between energy intake and expenditure. However, alterations in daily food intake and physical activity do not rapidly affect body weight. Why is this? The most plausible explanation is the existence of systems which maintain energy homeostasis throughout the body. Energy homeostasis is maintained by multiple mechanisms that involve gathering information on the body's nutritional status and making appropriate behavioral and metabolic responses to changes in fuel availability. For such inter-organ/tissue communication, humoral factors, including insulin and adipocytokines, are known to be very important. In addition, we and other research groups have recently reported the autonomic nervous system to play an important role in conveying metabolic information. Using these systems, the brain obtains information on peripheral metabolic status and processes it to send signals which regulate metabolism in the periphery. In particular, the hypothalamus is a primary site of convergence and integration for redundant energy status signaling, which includes central and peripheral neural inputs as well as hormonal and nutritional factors. These pathways of inter-tissue communication are summarized in Fig. 1. Recent advances in this field are reviewed herein.

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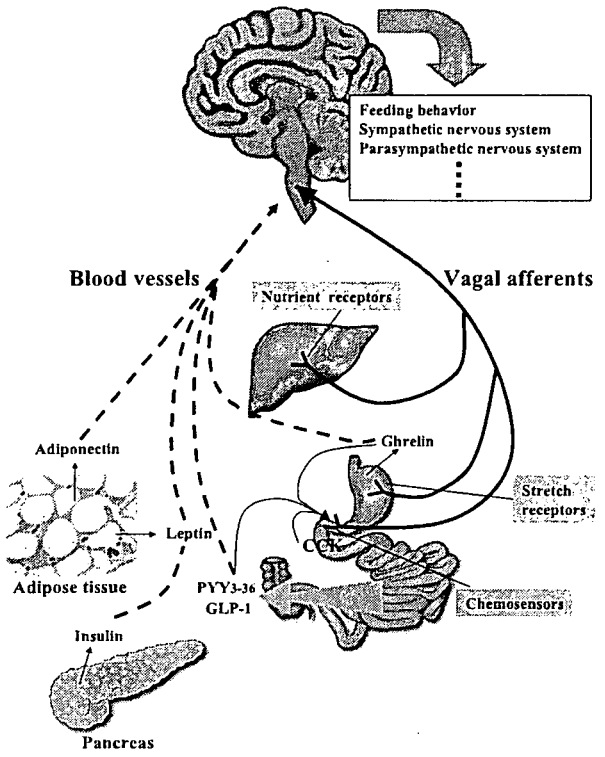


**Fig. 1.** Schematic presentation of inter-tissue communication (quoted from [31] with slight modification).

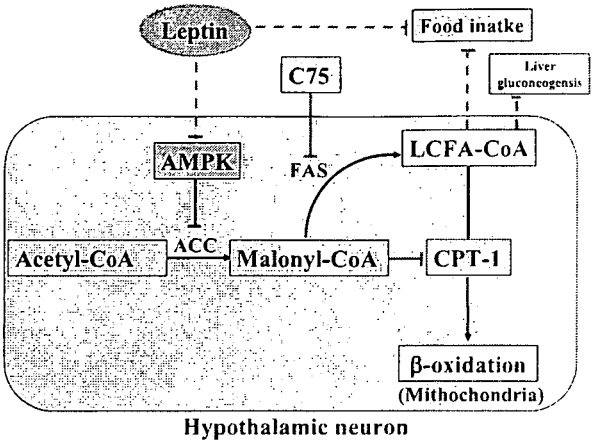


**Fig. 2.** Fatty acid metabolism, in the hypothalamic cells, which plays an important role in maintaining energy homeostasis (quoted from [65] with slight modification).

## 1. Brain inputs — humoral factors —

*1) Nutrients*

It is reasonable that essential nutrients, such as carbohydrates, lipids and proteins, mediate nutritional signals to the central nervous system by themselves. First, we will focus on the mechanism whereby these nutrients convey peripheral fuel status to the central nervous system.

*a) Free fatty acids*

The access of circulating free fatty acids to cerebrospinal fluids is generally proportional to the plasma fatty acid concentration [2, 3], indicating that the brain may acquire information regarding the peripheral metabolic state via cerebrospinal fatty acid levels. Fatty acid-sensitive neurons have been identified in the hypothalamus. For instance, an *in vitro* patch clamp study [4] showed that, among arcuate neurons, 13% of cells had increased electrical activity, while 6% had decreased activity when oleic acid was applied. Intracerebroventricular administration of oleic acid reportedly inhibits hepatic glucose production and food intake [5]. In addition, hypothalamic inhibition of carnitine:palmitoyl-CoA transferase-1 (CPT-1), an important mitochondrial enzyme that transfers long-chain fatty acyl-coenzyme A (LCFA-CoA) into mitochondria, decreases food intake and suppresses endogenous glucose production in the liver [6]. Furthermore, it was also reported that efferent vagal nerve signals from the brain to the liver are involved in hepatic gluconeogenesis in these experimental settings [7]. Hu *et al.* reported that central administration of C75, a potent inhibitor of fatty acid synthase (FAS), decreased food intake [8]. Since FAS inhibition increases malonyl-CoA and thus suppresses CPT1 activity, LCFA-CoA in hypothalamic neurons appears to be increased. Taken together, these results indicate that the cytosoplasmic LCFA-CoA concentration in hypothalamic neurons plays an important role in energy homeostasis (Fig. 2).

Leptin, an anorexigenic factor, reportedly decreases AMP-activated protein kinase (AMPK) activity in hypothalamic neurons [9], while ghrelin, an orexigenic factor, increases it [10]. AMPK is the downstream component of a kinase cascade that acts as a sensor of cellular energy charge, being activated by rising AMP coupled with falling ATP. AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC), resulting in decreased malonyl-CoA levels. As malonyl-CoA inhibits CPT-1, AMPK activation decreases cytoplasmic LCFA-CoA levels. Thus, cytoplasmic LCFA-CoA in

hypothalamic neurons may be involved in appetite regulation by leptin and ghrelin.

*b) Amino acids*

Amino acids also seem to communicate energy status information from the periphery. Transport of amino acids across the blood-brain barrier has been demonstrated [11]. The levels of amino acids in cerebrospinal fluids are reflected by peripheral blood levels. Central administration of leucine increases hypothalamic mTOR (mammalian target of rapamysin) activity, and thereby decreases food intake and body weight [12]. mTOR is a highly conserved serine/threonine kinase, present in organisms from yeast to mammals, the activity of which is sensitive to levels of branched-chain amino acids, especially L-leucine [13, 14]. Thus, mTOR is known to be one of the energy sensors for amino acids conserved in throughout evolution in organisms and, in mammals, hypothalamic mTOR signaling appears to play an important role in regulating systemic energy metabolism. Leptin increases hypothalamic mTOR activity, and the inhibition of mTOR signaling blunts leptin's anorectic effect [12], although further studies are needed to clarify the role of mTOR in energy homeostasis.

*c) Glucose*

It is well known that increases in serum glucose affect glucose-sensing neurons in the hypothalamus, resulting in suppression of food intake and liver gluconeogenesis (glucostatic theory). Two populations of glucose-sensing neurons have been defined: those excited (in which electrical activity is increased; GE neurons) and those inhibited (decreased activity; GI neurons) as local glucose levels rise. Such neurons have mainly been characterized in the ventromedial hypothalamic nucleus (VMH) and the arcuate nucleus (ARC) [15]. Glucose sensing mechanisms in pancreatic β cells, which secrete insulin in response to rising blood glucose, have been well analyzed. The glucose sensor in pancreatic β cells involves mainly GLUT2, glucokinase and specific KATP channels. Analogously, glucose sensing mechanisms in glucose-responsive neurons have been proposed. Two recent studies found GLUT2 expression in the rat brain [16, 17]. In addition, glucokinase is expressed in the rat hypothalamus [18]. Expressions of both GLUT2 and glucokinase have also been demonstrated in the human hypothalamus [19]. Using calcium imaging and single cell RT-PCR in freshly dissociated neurons from the VMH, Kang *et al.* confirmed the presence of glucokinase and KATP channels in some glucosensitive neurons [20]. KATP channel activity represents a key step in the electrical activity of GE neurons in the ARC and VMH in response to glucose concentration changes [21].

*2) Insulin*

Insulin is a product of pancreatic β cells and is the master metabolic switch between the fed and fasted states, mediating metabolic fuel disposition and use. Therefore, it has been proposed that insulin itself might be the fuel status signal to the brain, but the precise mechanisms have long been unclear. The activation of insulin signaling in the ARC, in the absence of elevated systemic insulin, is sufficient to decrease food intake and blood glucose levels via substantial inhibition of endogenous glucose production (EGP) [22, 23]. A recent study revealed the central effects of insulin on the suppression of EGP to be mediated by the insulin receptor-insulin receptor substrate 2 (IRS2)-phosphatidylinositol 3OH kinase (PI3K) pathway, resulting in KATP channel activation in the ARC [24]. Inoue *et al.* reported that centrally administered insulin induces IL-6 production in the liver, followed by STAT3 activation, resulting in suppression of hepatic EGP [25]. The activation of insulin receptors in the brain, in particular the ARC of the hypothalamus, plays an important role in the regulation of glucose homeostasis and food intake.

*3) Adipocytokines*

*a) Leptin*

Leptin is produced mainly in adipocytes in proportion to fat stores; adequate leptin levels communicate the repletion of body energy stores to the central nervous system in order to suppress food intake and permit energy expenditure [26]. Leptin binds to leptin receptors (Ob-Rb) in the hypothalamus, resulting in activation of the JAK/STAT pathway [27, 28] and the IRS2/PI3K pathway [29]. In addition, Minokoshi *et al.* recently reported that leptin suppressed hypothalamic AMPK activity, leading to food intake suppression [9]. As stated above, leptin also activates mTOR signaling in the hypothalamus. Thus, leptin signaling involves at least four pathways, JAK/STAT, IRS2/PI3K, AMPK and mTOR. Complicated interactions

may exist among these four pathways.

In most individuals with ordinary obesity, circulating leptin levels are elevated, but the body does not adequately respond to this increased leptin with reduced food intake. This under-responsiveness to leptin in most forms of obesity has given rise to the idea that obesity is associated with, or even caused by, a state of relative leptin resistance similar to insulin resistance. The mechanisms underlying leptin resistance remain a matter of debate. From the therapeutic point of view, the mechanism underlying leptin resistance is an important issue which awaits clarification.

*b) Adiponectin*

There is a recent report [30] suggesting adiponectin to have central effects on energy metabolism. Intravenous administration of adiponectin increased the level in cerebrospinal fluid. In addition, central adiponectin administration increased systemic energy expenditure and reduced body weight, followed by decreased blood glucose and serum lipid levels. Detailed studies are needed to clarify the roles of adiponectin in communicating the peripheral metabolic state to the central nervous system.

## 2. Brain inputs — afferent nerve signals —

*1) Innervation*

*a) Intra-abdominal innervation without white adipose tissues*

First, the innervation of intra-abdominal tissues requires explanation. For example, the gut is innervated by both splanchnic (sympathetic) and vagal (parasympathetic) nerves. Detailed fiber count studies have revealed that the abdominal vagal nerve is comprised of approximately 75% afferent fibers, the splanchnic nerve 50%. Afferent signals from the gut to the brain are carried in vagal and splanchnic nerve pathways. Vagal afferents respond to specific luminal chemical stimuli, physiological levels of distention or nutrients, whereas splanchnic afferents convey information regarding noxious stimuli [31]. On the other hand, intrapelvic organs, urogenital organs and so on, are innervated by a pelvic nervous plexus, which consists of both sympathetic and parasympathetic nerves.

*b) Innervation of intra-abdominal adipose tissues*

White adipose tissues are also innervated by both efferent and afferent nerve fibers. As for the efferent sympathetic fibers, numerous reports have described functions, including lipolysis or β oxidation [32–34]. On the other hand, a recent study demonstrated white adipose tissues to be innervated by efferent parasympathetic nerve fibers [35], although their physiological functions remain to be elucidated. There are only a few articles focusing on the functions of afferent nerve fibers from white adipose tissues. Niijima [36] and Tanida *et al.* [37] used electrical firing measurements to show that leptin induces functional activation of afferent nerve fibers from epididymal white adipose tissues. In a more recent study, afferent nerve innervation in epidydimal white adipose tissues was demonstrated anatomically [38]. In addition, we recently reported the functional significance of afferent nerve signals from intra-abdominal adipose tissues which modulate hypothalamic leptin sensitivity, as described in detail below [39].

*2) Signals transmitted by afferent autonomic nerve fibers*

*a) Signals from the gut*

It has been reported that afferent autonomic nerve fibers convey signals carrying information about energy homeostasis [40–43]. Physiological distention of the gut as well as cholecystokinin (CCK) [44], PYY3-36 [45] and glucagon-like peptide-1 (GLP-1) [46] stimulate afferent vagal nerve fibers, resulting in food intake suppression. In contrast, ghrelin enhances food intake via the afferent vagus [47]. CCK is produced by mucosal enteroendocrine cells of the duodenum and jejunum and is secreted in response to the presence of food within the gut lumen. Sulfated CCK, which preferentially binds to CCK1 receptors on vagal afferent neurons, sends satiety signals to the brain; hence, vagotomy inhibits the anorectic effect of CCK [44]. GLP-1 and PYY3-36 secretions from enteroendocrine L cells are triggered by luminal nutrients. The mechanisms by which sugars activate L cells involve the closure of ATP sensitive potassium channels, resulting in depolarization of the cells, via a mechanism analogous to insulin secretion from β cells [48, 49]. Koda *et al.* [45] showed that peripheral administration of PYY3-36 stimulates vagal afferent nerves via a Y2 receptor which is expressed at nerve terminals. Abdominal

vagotomy abolished the anorectic effect of PYY3-36. Similarly, the anorectic effects of peripheral GLP-1 administration were also abolished by vagotomy [46]. Thus, peripheral PYY3-36 and GLP-1 transmit satiety signals to the brain via the vagal afferent pathway. On the other hand, ghrelin is a peptide recently found to be produced in the stomach, which acts on a previously identified orphan receptor (growth hormone secretagogue receptor), activation of which in the hypothalamus causes growth hormone (GH) release from the pituitary gland [50]. Date *et al.* [47] reported blockade of the gastric vagal afferent to abolish ghrelin-induced feeding, GH secretion and the activations of NPY- and GHRH-producing neurons. The ghrelin receptor is also expressed in vagal afferent terminals, and ghrelin suppresses vagal afferent firing. Taken together, these findings indicate involvement of gastric vagal afferent in conveying signals regarding satiety as well as starvation from the gut to the brain.

*b) Signals from the liver — Liver functions as an energy balance sensor —*

*(1) Hepatoportal glucose sensor*

Nutrients absorbed from the gut enter the portal vein, and thereby reach the liver directly. Therefore, given its anatomical location, it seems reasonable that the liver functions as a glucose sensor. It has been demonstrated that signals regarding serum glucose levels from the so-called hepatoportal glucose sensor to the brain are carried along afferent vagal nerve pathways [40]. The hepatoportal glucose sensor, which is an as yet incompletely defined structure, is activated by a glucose gradient established between the portal vein and the periphery. Raising portal vein glucose levels leads to a decrease in vagal afferent discharges reaching the nuclei of solitary tract neurons, leading to activation of sympathetic efferents to the adrenal glands, liver, splanchnic bed and pancreas. Because all of these reflex efferent outputs are blocked by hepatic vagotomy, it appears that signals triggered by high levels of portal glucose are transmitted through vagal afferents [51, 52]. Burcelin *et al.* showed that the hepatoportal sensor requires the presence of GLUT2 but that hepatocytes are not involved in this sensing process [53], in agreement with previous studies showing this sensor to be located upstream from the hepatic hylus [54]. They also reported that GLP-1 signaling modulates hepatoportal glucose sensing [55], an observation compatible with the role of GLP-1 in regulating the firing activity of hepatic vagal afferents [56]. A similar role for GLP-1 in canine hepatoportal sensor function has also been reported [57].

On the other hand, sympathetic afferents mediate hypoglycemic signals. Reportedly, a counterregulatory response to moderate systemic hypoglycemia, *i.e.* sympathetic efferent activation, is attenuated by clamping the liver at euglycemic levels and is disrupted by interruption of sympathetic (but not vagal) afferents from the hepatic portal circulation [58, 59]. Collectively, these observations indicate that the afferent autonomic nervous system, including both vagal and sympathetic nerves, from the hepatoportal structure, plays important roles in conveying information regarding peripheral glucose levels to the brain.

*(2) PPARγ (peroxisome proliferator-activated receptor γ)*

Recently, in a number of studies, tissue-specific knockout mice have been found to exhibit unexpected phenotypes, suggesting the presence of as yet unknown cross-talk between organs/tissues. Therefore, unraveling the complexities of this inter-organ communication would be very important for elucidating the mechanisms underlying not only energy and glucose homeostasis but also the development of obesity-related diseases. However, using genetically engineered mice, it is somewhat difficult to demonstrate the underlying mechanisms, since a substantial number of compensatory mechanisms can modify metabolic phenotypes. Alternatively, using adenoviral gene transfer into an organ/tissue of an adult mouse model, we observed an example of such inter-tissue communication; dissipating excess energy in the liver affects insulin sensitivity in muscle and adipose tissues [60]. Therefore, we suspected that, if metabolism could be altered in just one organ, it would be easier to analyze acute metabolic effects in other remote tissues and, assuming intervention to be possible, it would give us an understanding of the mechanisms.

Mice with tissue-specific knockout of peroxisome proliferator-activated receptor γ (PPARγ) may provide an example of such inter-tissue communication. Notably, liver-specific disruption of PPARγ in ob/ob mice prevented hepatic steatosis, but increased peripheral adiposity and decreased insulin sensitivity in muscle and adipose tissue [61]. Hepatic expression of PPARγ, especially PPARγ2, is functionally enhanced in a number of obesity models [62, 63]. Therefore, to unravel the mechanism underlying this inter-organ/tissue com-

munication between the liver and peripheral tissues including muscle and fat, we overexpressed PPARγ2 in the livers of mice using adenoviral gene transfer.

Hepatic PPARγ2 expression acutely induced severe hepatic steatosis, while peripheral adiposity was markedly reduced due to enhanced lipolysis. Systemic metabolic rates were increased and, therefore, peripheral insulin sensitivity and glucose tolerance showed marked improvement. Thus, hepatic expression of PPARγ2 exerts not only local effects in the liver, but also remote effects in adipose tissues and the whole body. These remote effects were explained by increased sympathetic outflow into muscle and adipose tissues. Therefore, to examine the possibility that afferent nerves originating in the liver are involved in the observed effects on energy expenditure and peripheral adiposity through efferent sympathetic nerve activation, we interrupted liver-brain communication by performing selective hepatic branch vagotomy. This manipulation significantly reversed the both reduction in peripheral adiposity and the enhancement of energy expenditure. In addition, pharmacological deafferentation of the vagus blocked the hepatic PPARγ2 expression-induced decrease in white adipose tissue weights. These findings indicate that hepatic PPARγ2 expression and/or hepatic lipid accumulation stimulate afferent vagal nerve fibers, communicating metabolic information to the brain, leading to anti-obesity and anti-insulin-resistant effects in muscle and adipose tissue [64].

Fat storage in the liver changes dynamically according to the systemic energy balance and is associated with several features of the metabolic syndrome. Since hepatic PPARγ expression is physiologically associated with obesity, the liver may convey information regarding excess energy to the central nervous system via the afferent vagus. This neuronal system is likely to underlie chronic "adaptive thermogenesis", resulting in protection against metabolic perturbation induced by excessive energy storage (Fig. 3). When the brain obtains information regarding excess energy storage mediated by leptin from adipose tissues and via the afferent vagus from the liver, the sympathetic nervous system is activated to enhance energy expenditure and lipolysis, thereby maintaining energy homeostasis.

*c) Signals from adipose tissues*

There are only a few reports focusing on afferent nerve signals from adipose tissue. According to these reports, activation of afferent nerves from intra-abdominal (epididymal) adipose tissue resulted in reflex signals being sent to white adipose tissues via efferent sympathetic nerve activation [36, 37]. However, the functional significance of these afferent signals was unclear. We demonstrated hypothalamic leptin sensitivity to be modulated through afferent nerves from epididymal fat [39].

Fat accumulation in intra-abdominal fat tissue plays a major role in development of the metabolic syndrome associated with insulin and leptin resistance. Leptin resistance is induced by excessive adiposity and, in turn, is an important mechanism underlying maintenance of the obese state. To determine whether a local reduction in the adiposity of intra-abdominal adipose tissue of diabetic mice with diet-induced obesity would reverse obesity-related metabolic disorders, in particular insensitivity to leptin and insulin, we attempted to express uncoupling protein-1 (UCP1), which functions to dissipate energy as heat. UCP1 was expressed in epididymal adipose tissue only at very low levels. Nevertheless, food intake declined in association with decreased serum leptin levels as well as downregulation of the orexigenic neuropeptide Y and upregulation of the anorexigenic precursour neuropeptide proopiomelanocortin, in the hypothalamus. The anorectic response to exogenous leptin was enhanced by adipose UCP1 expression. In addition, the hypophagia could not be duplicated in db/db mice with mutant leptin receptors. Collectively, these findings clearly show that very limited UCP1 expression in the intra-abdominal fat pad dramatically improves hypothalamic leptin resistance. Local dissection of nerves from the epididymal fat pad and pharmacological deafferentation blunted the anorectic effects of UCP1 expression in adipose tissue. Taken together, the results suggest afferent nerve signals originating in epididymal fat pads to modulate hypothalamic sensitivity to leptin (Fig. 4).

Adipose tissues were long regarded as a simply being passive fuel storage sites. However, the discovery of various adipocytokines, with leptin being the most important example, has raised adipose tissue to the status of a versatile endocrine gland. Recent studies including ours provide further evidence of the key role of adipose tissue as a base from which neuronal signals regulating feeding and fuel metabolism are sent. Furthermore, identification of the neurotransmitted

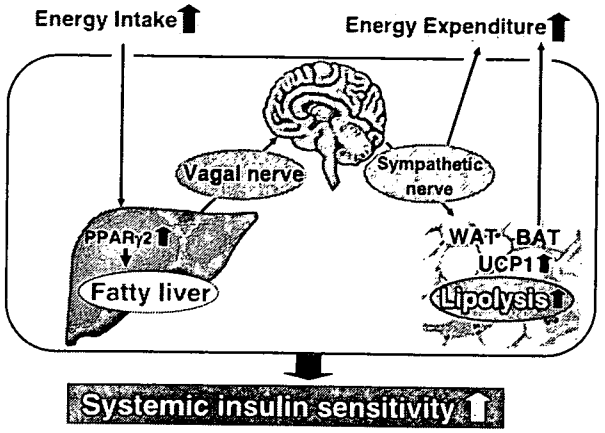


Fig. 3. Scheme of the neuronal pathway originating in the liver. Hepatic PPARγ expression associated with surplus energy results in increased energy expenditure, decreased peripheral adiposity and improved insulin sensitivity via the neuronal system consisting of afferent vagal and efferent sympathetic nerves.

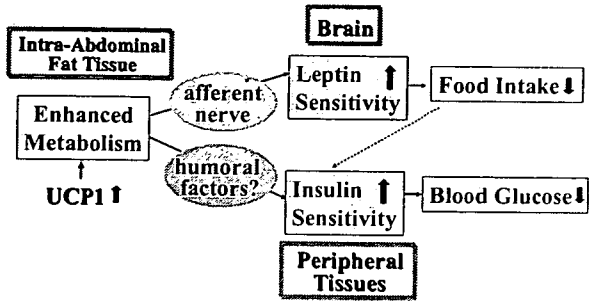


Fig. 4. The proposed mechanism whereby UCP1 expression in epididymal fat tissue decreases food intake and improves glucose tolerance (quoted from [39] with slight modification).

substance involved might lead to development of novel therapeutic strategies aimed at tackling the metabolic syndrome.

## Conclusion

Metabolism does not go on independently in different organs/tissues, but rather in a coordinated and regulated manner throughout the body. Metabolic regulation coordinated among organs/tissues, which requires communication among these organs/tissues, appears to be essential for maintaining the homeostasis of systemic metabolism, in particular glucose and energy metabolism. In addition, disturbance of this coordinated control system may be implicated in the development of metabolic disorders, such as obesity, type 2 diabetes, hyperlipidemia and the metabolic syndrome.

Recent advances in this field revealed the complex and important roles of the central nervous system. The brain obtains a variety of metabolic information from peripheral organs/tissues through humoral and neuronal avenues. These inputs are likely to be integrated and processed in the brain, leading to the transmission of regulatory signals, which induce appropriate metabolic responses, throughout the body. Further elucidation of these regulatory systems, in much greater detail, may allow us to unravel the mechanisms underlying metabolic homeostasis and thereby to understand the metabolic disorders. Moreover, targeting of these neuronal pathways is a potential therapeutic strategy for the metabolic syndrome.

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Current Topics

## Metabolism and Functions of Phosphoinositides

# Role of Phosphatidylinositol 3-Kinase Activation on Insulin Action and Its Alteration in Diabetic Conditions

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**Inositol phospholipids phosphorylated on D3-position of their inositol rings (3-phosphoinositides) are known to play important roles in various cellular events. Activation of PI (phosphatidylinositol) 3-kinase is essential for aspects of insulin-induced glucose metabolism, including translocation of GLUT4 to the cell surface and glycogen synthesis. The enzyme exists as a heterodimer containing a regulatory subunit and one of two widely-distributed isoforms of the p110 catalytic subunit: p110$\alpha$ or p110$\beta$. Activation of PI 3-kinase and its downstream AKT has been demonstrated to be essential for almost all of the insulin-induced glucose and lipid metabolism such as glucose uptake, glycogen synthesis, suppression of glucose output and triglyceride synthesis as well as insulin-induced mitogenesis. Accumulated PI(3,4,5)$P_3$ activates several serine/threonine kinases containing a PH (pleckstrin homology) domain, including Akt, atypical PKCs, p70S6 kinase and GSK.**

**In the obesity-induced insulin resistant condition, JNK and p70S6K are activated and phosphorylate IRS-proteins, which diminishes the insulin-induced tyrosine phosphorylation of IRS-proteins and thereby impairs the PI 3-kinase/AKT activations. Thus, the drugs which restore the impaired insulin-induced PI 3-kinase/AKT activation, for example, by suppressing JNK or p70S6K, PTEN or SHIP2, could be novel agents to treat diabetes mellitus.**

**Key words** insulin; PI 3-kinase; diabetes mellitus; insulin resistance

## 1. INTRODUCTION

The insulin receptor is a transmembrane glycoprotein that mediates the first step in insulin action. When insulin binds to the extracellular domain of the receptor, the intrinsic tyrosine kinase activity of the intracellular domain of the receptor is activated. Then, the activated insulin receptor tyrosine kinase phosphorylates several tyrosine residues of the insulin receptor itself, as well as several intracellular substrates. This review describes how these intracellular substrates transmit the signals necessary to induce various metabolic actions of insulin.

Insulin resistance plays a major role in the occurrence and development of Type 2 diabetes mellitus, which accounts for over 85% of diabetes worldwide. As insulin resistance develops, pancreatic $\beta$-cells compensate by secreting more insulin until their capacity to produce adequate amounts of the hormone is exhausted, and the elevation of blood glucose becomes manifest.

Although very probably there are many genetic factors affecting insulin sensitivity in humans, it is well-known that insulin sensitivity is also affected by environmental factors closely linked to modern civilization, by affluence and by increased life expectancy. All of the aforementioned factors contribute to insulin resistance, and that they reflect a variety of molecular mechanisms. There have been a number of recent studies investigating the intracellular signaling pathway leading from the binding of insulin to its receptor to activation of glucose and lipid metabolisms.

## 2. INSULIN-INDUCED PI 3-KINASE ACTIVATION IS MEDIATED BY IRS-PROTEINS

The principal insulin receptor substrates, called IRS-proteins, are phosphorylated on multiple tyrosine residues by the activated insulin receptor. The IRS-protein family consists of at least 4 isoforms, termed IRS-1, 2, 3 and 4.[1] These IRS-proteins possess a PH domain and a PTB domain at their N-termini, and associate with the insulin receptor *via* these domains. Phosphorylated IRS-proteins recruit various signaling proteins into a multicomponent complex through the interaction between their phosphotyrosine-containing motifs and the SH2 domain of downstream signaling molecules. These SH2-containing proteins include the regulatory subunit of the PI 3-kinase, Grb2, SHP-2, fyn, and others.[1] In addition, it was shown that 14-3-3 protein recognizes and binds to the phosphorylated serine containing motif of IRS-proteins.[2] This association with 14-3-3 protein inhibits the association of IRS-1 with the insulin receptor and resultant tyrosine phosphorylation. Thus, this mechanism may be important for regulating the functions of IRS-proteins, particularly transfer of the signal from the insulin receptor to downstream factors. Although other SH2 proteins also bind to IRS-1/2, PI 3-kinase is considered to be particularly important for the insulin-induced glucose uptake into muscle and adipose tissue, which is dependent on the translocation of a glucose transporter to the plasma membrane. Indeed, the overexpression of constitutively-active, membrane-targeted or GLUT2-tagged p110$\alpha$ increased translocation of GLUT4 glucose

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transporter to the cell surface and increased basal glucose transport, irrespective of the presence of insulin.[3—5] Treatment with specific inhibitors of PI 3-kinase or overexpression of the dominant negative form of the PI 3-kinase regulatory subunit inhibited insulin-induced glucose uptake, glycogen synthesis and DNA synthesis. In addition, PI 3-kinase activation also reportedly plays an important role in insulin-induced glycogen synthesis and suppression of phosphoenolpyruvate carboxykinase (PEPCK) expression in hepatocytes.[6—8] Thus, PI 3-kinase activation is considered to play a critical role in insulin-induced glucose metabolism in muscle, fat, and liver.

Although many growth factors and hormones similarly activate PI 3-kinase, it should be noted that insulin is the only hormone which effectively induces glucose metabolism. This difference can be explained as followings; insulin-induced activated PI 3-kinase is translocated to the specific intracellular place with the associated IRS-1 or IRS-2, while many growth factor receptors such as PDGF (platelet derived growth factor) receptor induces the activation of PI 3-kinase at the plasma membrane with a direct association of PI 3- kinase with the receptor on the plasma membrane. Thus, such different location in the activated PI 3-kinase is considered to induce the different response for the glucose metabolism. Furthermore, interestingly, IRS-1 and IRS-2 reportedly exhibit the different subcellular distribution although not shown very clearly,[9] and analysis using IRS-1 and IRS-2 KO mice demonstrated that IRS-1 is important for insulin-induced metabolism in the muscle and adipose tissue, while IRS-2 in the liver and pancreatic $\beta$ cells.[10]

## 3. STRUCTURE OF PI 3-KINASES

PI 3-kinase exists as a dimer composed of a 110-kDa catalytic subunit associated with a regulatory subunit (Fig. 1). Activated receptors with tyrosine kinase activity often interact with regulatory subunit SH2 domains *via* phosphorylated YXXM motifs,[11] in turn activating or recruiting PI 3-kinase. The regulatory subunit contains two proline-rich motifs (PRMs), two SH2 domains and a domain (IS) situated between the SH2 domains, which is responsible for binding to p110.[12] To date, five isoforms of the regulatory subunit have been identified: two 85-kDa proteins (p85$\alpha$, p85$\beta$),[12,13] two 55-kDa proteins (p55$\alpha$, p55$\gamma$)[14—16] and one 50-kDa protein (p50$\alpha$).[17] Interestingly, transcription of the p85$\beta$ gene yields three isoforms of the regulatory subunit. The p85$\beta$ and p55$\gamma$ genes, by contrast, express single 85-kDa and 55-kDa protein products, respectively. Although lacking the Src-homology 3 (SH3) and BCR homology (BH) domains, a similar short form of the regulatory subunit was found in drosophila (droPIK57).[18] Such conserved expression among distant species is likely indicative of the importance of the short forms of the regulatory subunits. Structural differences in the N-terminal regions of the different group members contribute to defining their binding specificity, their subcellular distributions and their capacity to activate the 110-kDa catalytic subunit.

Two widely distributed isoforms of the catalytic subunit have been identified as p110$\alpha$ and p110$\beta$. On the basis of *in vitro* assays of the lipid kinase activities of p110$\alpha$ and p110$\beta$ expressed in Sf-9 cells, basal p110$\alpha$ activity is substantially lower than that of p110$\alpha$. However, p110$\beta$ appears to be highly insulin-sensitive, while p110$\alpha$ was unaffected by insulin, despite the fact that both isoforms bind to the p85$\alpha$ subunit and to IRS-1 with similar efficiency.[19]

PI 3-kinase catalyzes the formation of PI-3-P, PI-3,4-$P_2$ and PI-3,4,5-$P_3$ from PI, PI-4-P and PI-4,5-$P_2$, respectively. Signaling molecules binding to PI-3,4-$P_2$ and PI-3,4,5-$P_3$ include serine/threonine kinases (PKB, PKC, PDK-1)[20—22]; guanine nucleotide exchange factors for small GTP-binding proteins (Grp1, Cytohesin1, ARNO, SOS, Tiam-1)[23—26]; protein tyrosine kinases (Src, Btk)[27,28]; and Clathrin adaptors (AP2, AP3).[29,30] Among them, Akt (also known as PKB), a serine/threonine kinase, translocates to the membrane frac-

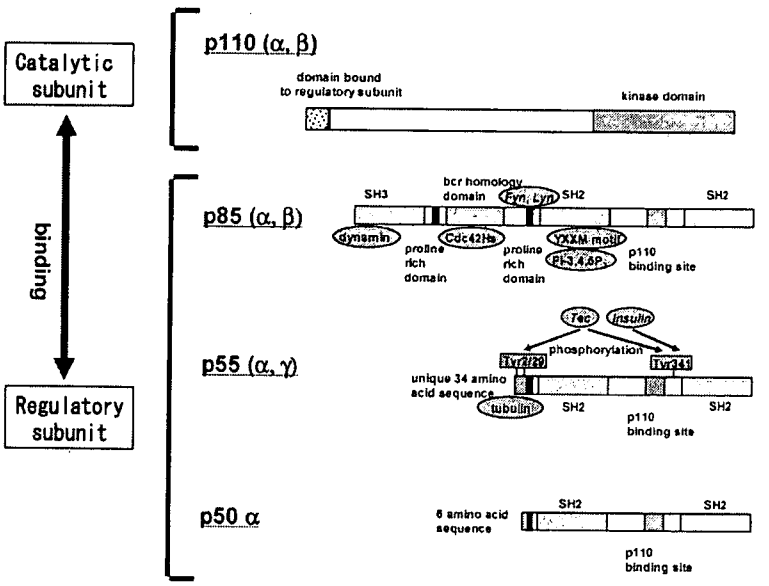


Fig. 1. Structure of the p85/p110 Type PI-Kinase
Schematic diagram showing the three classes of PI 3-kinase regulatory subunit (p85, p55 and p50) and the catalytic subunit (p110). Ellipses denote ligands binding to their respective binding sites on the subunits, while rectangles denote potential tyrosine phosphorylation sites.

tion *via* binding of the PH domain with the PI-3,4,5-$P_3$ produced by PI 3-kinase, and phosphorylations of Thr306 and Ser473 of Akt by PDK1[31] and Rictor/mTOR complex[32] take place thereby activating Akt kinase. This step was found to mediate various insulin- and growth factor-induced cellular responses, such as the stimulation of GLUT4 translocation to the plasma membrane, the inhibition of glycogen synthase kinase-3 (GSK-3), induction of triglyceride synthesis *via* increasing the expression of SREBP1C and the promotion of cell survival by inhibiting apoptosis.[33] Indeed, these cellular activities have been shown to be induced by overexpression of the constitutively active or the membrane targeted mutant of Akt, while Akt2-deficient mice show impaired glucose tolerance due to decreased insulin-induced glucose uptake in skeletal muscle and increased hepatic glucose production.[34] Thus, PI 3-kinase/Akt pathway is critically important for insulin-induced glucose and lipid metabolisms.

## 4. ALTERED INOSITOL PHOSPHOLIPID METABOLISM IN THE INSULIN RESISTANT CONDITIONS

Insulin resistance plays a major role in the occurrence and development of Type 2 diabetes mellitus, which accounts for over 85% of diabetes worldwide. As insulin resistance develops, pancreatic $\beta$-cells compensate by secreting more insulin until their capacity to produce adequate amounts of the hormone is eventually exhausted, and blood glucose elevation manifests.

It is well-known that insulin sensitivity is also affected by environmental factors closely linked to modern civilization, as well as mostly unidentified genetic factors. Once diabetes mellitus worsens, prolonged hyperglycemia is itself a factor diminishing insulin sensitivity. Consequently, any individual case of Type 2 diabetes mellitus will usually involve two or more factors reducing the patient's insulin sensitivity. For example, excess caloric intake, high-fat diet, insufficient exercise, aging and so on all contribute to the insulin resistance observed during the early, impaired glucose tolerance (IGT) stage of Type 2 diabetes mellitus; later in the disease process, when hyperglycemia is chronic, the contribution made by elevated blood glucose may be significant.

In a later portion of this review, we describe how insulin signaling is affected by factors such as obesity with overeating, high-fat diet, hyperglycemia, and salt-related hypertension, citing results obtained using rodent models.

**Impared PI 3-Kinase Activation in Zucker Fatty Obese Rats** As a first step, we analyzed the impaired insulin signaling in Zucker fatty rats, which are considered to be an excellent model of early-stage Type 2 diabetes mellitus induced by overeating and overweight. Zucker rats exhibit marked hyperinsulinemia and obesity due to a leptin receptor mutation, but they have relatively mild hyperglycemia. This constellation of symptoms is very similar to that observed in the early phase of human Type 2 diabetes mellitus. The insulin resistance in Zucker fatty rats involves both impaired glucose transport in muscle and impaired suppression of glucose production in the liver.

Some studies have suggested that insulin receptor tyrosine kinase activity is impaired in the Zucker fatty rat.[35] Our results, however, suggest that the degree of impairment at this step is relatively mild, which is in agreement with the results obtained with skeletal muscles from obese non-diabetic human subjects. A more significant factor contributing to insulin resistance in Zucker fatty rats is likely to be diminished expressions of IRS-1 and IRS-2.[36] Expressions of both IRS-1 and IRS-2 mRNA and protein were found to be downregulated in both liver and muscle of Zucker fatty rats. Moreover, it appears that mRNA transcription is more severely affected than protein synthesis. Consistent with decreased synthesis, insulin-induced increases in tyrosine phosphorylation of IRS-1 and IRS-2 were reduced in both liver and muscle of Zucker fatty rats. By normalizing the tyrosine phosphorylation of IRS-1/2 per unit protein, the decline in tyrosine phosphorylation was confirmed to be due mainly to the decline in the levels of these two proteins; although with the exception of IRS-2 in muscle, impaired insulin receptor-mediated phosphorylation also played a minor role.

Tyrosine phosphorylated IRS-1/2 binds to the regulatory subunit (*e.g.*, p85$\alpha$) of PI 3-kinase, thereby activating the enzyme. The insulin-induced increases in the amount of IRS-1/2-bound p85$\alpha$ and the corresponding increases in PI 3-kinase activity were all decreased in the livers and muscles of fatty rats.[36] Of particular importance to us was the finding that decreases in hepatic PI 3-kinase activity were more marked than the decreases in tyrosine phosphorylation or in the binding of IRS-1/2 to p85$\alpha$.

The association of IRS-1/2 with PI 3-kinase occurs *via* phosphorylated YMXM or YXXM motifs on IRS-1/2 and the SH2 domains on the regulatory subunit of PI 3-kinase (Backer *et al.* 1992). For full activation of PI 3-kinase, it is necessary for both SH2 domains to be bound; binding of only one SH2 domain to a phosphorylated motif induces only partial activation. Thus, IRS-1/2 must be phosphorylated on two or more tyrosine residues in the Y(M/X)XM motifs to fully activate the associated PI 3-kinase, even though IRS-1/2 containing only a single phosphorylated Y(M/X)XM motif can bind to the enzyme. In addition, tyrosine phosphorylation of sites other than the Y(M/X)XM motifs would not contribute to the activation of PI 3-kinase, though they may be recognized by our anti-phosphotyrosine antibody. The discrepancy between the levels of IRS-1/2 tyrosine phosphorylation and the associated PI 3-kinase activities, therefore, may be explained by a selective decrease in phosphorylation of the tyrosine residues in Y(M/X)XM motifs. However, the intramolecular mechanisms regulating ty-

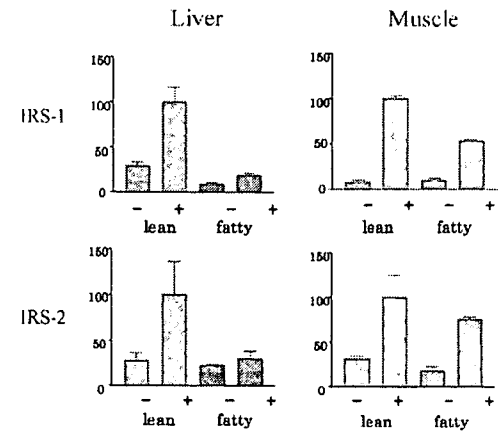


Fig. 2. Impaired p85/p110 Type PI-Kinase Activation in Zucker Fatty Rats