Effects of Food Restriction on Peroxisome Proliferator–Activated Receptor-γ and Glucocorticoid Receptor Signaling in Adipose Tissues of Normal Rats

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In adipocytes, peroxisome proliferator–activated receptor (PPAR)-γ activates adipocyte differentiation and glucocorticoid (GC) stimulates the expression of PPAR-γ mRNA. The local tissue concentrations of GC, in turn, are modulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). To clarify the change of energy metabolism in condition of reduced energy intake, we investigated whether food restriction alters the adipocyte size and levels of PPAR-γ, GC receptor (GR), and 11β-HSD1 mRNA expression in the white adipose tissues of normal rats. Male Wistar rats weighing 340 g were housed under free feeding or 20% reduction of food intake for 2 or 14 days. We found that 2-day food restriction did not cause any change in the mean size or number of adipocytes in the omentum, while 14-day food restriction decreased the size and increased the number of adipocytes. In addition, the levels of PPAR-γ2, GR, and 11β-HSD1 mRNA expression in the omentum were lower in the food-restricted rats after 2 days, while they did not differ after 14 days. Also, after both 2 and 14 days, plasma concentrations of free fatty acid (FFA) were higher in the food-restricted rats than in control rats. Finally, plasma concentrations of adrenocorticotropin (ACTH) and corticosterone were the same in the both groups after 2 days, although they were higher in the food-restricted rats after 14 days. These results suggest that adipocyte differentiation in the omentum of food-restricted rats is attenuated after 2 days but recovers after 14 days, resulting in an increase in the number of small adipocytes. It is also likely that lipolysis induced during the 14-day period of food restriction decreased the size of adipocytes. Further, food restriction may affect the efficiency of local GC effects by altering GR and 11β-HSD1 mRNA expression. Also, higher levels of plasma GC and recovery of GR and 11β-HSD1 mRNA expression may contribute to the recovery of the levels of PPAR-γ2 mRNA expression in the omentum and result in the recovery of adipocyte differentiation.

THE DEVELOPMENT of obesity consists of 2 processes: adipocyte differentiation, the formation of new adipocytes from the stroma cells, and adipocyte hypertrophy, an increase in adipocyte size due to fat storage. The expression of the numerous genes, such as members of peroxisome proliferator–activated receptor (PPAR)-γ, adipocyte determination- and differentiation-dependent factor 1/sterol response element-binding protein 1, CCAAT/enhancer-binding protein nuclear receptor families, adipocyte complement-related protein of 30 kd (Acrp30)-AdipoQ, and adipsin, is increased in the process of adipocyte differentiation.[1-5]

PPAR-γ is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily and is abundantly expressed in both white and brown adipose tissues.[6] It is induced early in the process of the adipocyte differentiation and has a critical role for this through activation of adipose-specific genes.[6,7] There are 2 isoforms of PPAR-γ, referred to as PPAR-γ1 and PPAR-γ2. These 2 forms of PPAR-γ are derived from the same gene by alternative promoter usage and differential mRNA splicing.[2,8] Both PPAR-γ1 and PPAR-γ2 mRNA are abundantly expressed in adipose tissue.[9] Thiazolidinediones (TZDs), ligands for PPAR-γ, increase the action of insulin[10] and induce the differentiation of the adipocytes of normal and obese rats.[11] Glucocorticoid (GC) and insulin synergistically induce the expression of PPAR-γ mRNA in isolated human adipocytes and 3T3-L1 cells.[12,13]

The biological activity of GC can be determined by the number and affinity of GC receptor (GR) and the amount and activity of 11β-hydroxysteroid dehydrogenase (11β-HSD) in the tissues. 11β-HSD catalyzes the interconversion of hormonally active C11-hydroxylated corticosteroids (cortisol, corticosterone) and their inactive C11-keto metabolites (cortisone, 11-dehydrocorticosterone). Two isoforms of 11β-HSD have been identified, 11β-HSD type 1 (11β-HSD1) and 11β-HSD type 2 (11β-HSD2), which differ in their biological properties and tissue distributions. 11β-HSD1 predominantly changes inert GC to their active forms and maintains high levels of GC in tissues such as liver and adipose tissue.[14]

Improvements in intermediary metabolism and reduction in risk factors for coronary heart disease accompany the selective reduction of visceral adiposity through diet and exercise.[15,16] However, it is sometimes difficult to sustain the treatment over a long period of time, as shown by the attenuation of weight loss or regain of body weight.[17] These phenomena may be induced by metabolic adaptation for mild restriction of calorie intake. However, the mechanism of these phenomena is not fully understood. We therefore performed this study to clarify the mechanism of the adaptation for mild calorie restriction. We suspected that the levels of PPAR-γ, GR, and 11β-HSD1 mRNA expression in white adipocytes may be changed, and that the differentiation of adipocytes may be induced by these changes during mild food restriction in normal rats. We also examined the levels of uncoupling protein 1 (UCP1) mRNA expression in brown adipose tissue to determine whether UCP1, which plays a role in energy expenditure by inducing nonshivering thermogenesis in brown adipose tissue,[18,19] contributes to changes in energy metabolism during mild reduction of energy intake.

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MATERIALS AND METHODS

Animals

Male Wistar rats weighing 340 to 350 g were housed under conditions of controlled temperature and illumination (8 AM to 8 PM). Animals were maintained according to the guidelines for Animal Experimental Ethical Review Committee of Nippon Medical School. Body weight and daily food intake were measured at 9 AM every day.

Food Restriction and Collection of Blood Samples and Adipose Tissues

Rats were given a standard diet containing 50.7% carbohydrate, 24.9% protein, and 4.6% fat (Crea Japan, Tokyo, Japan). Twelve rats were freely fed as control and another 12 rats were restricted to 80% of the daily food intake of control rats. After 2 and 14 days, 6 rats of each group were killed by decapitation and truncal blood samples were collected between 9 AM and 11 AM. Following centrifugation, plasma samples were stored at −20°C until use. The omentum, subcutaneous and brown adipose tissues were immediately removed when rats were killed and stored at −80°C. Total RNA was extracted from each tissue by the guanidium thiocyanate-phenol-chloroform method using Isogen (Nippon Gene Co, Toyama, Japan).[20]

Histological and Morphological Study of the Adipose Tissues

Omentum and subcutaneous adipose tissues were fixed with 6.7% formalin and embedded in paraffin. Two-micron paraffin sections were cut and stained with hematoxylin and eosin for light microscopic examination. Four unit areas (545 × 545 μm per unit area) were randomly chosen for each adipose tissue and the size and number of the adipocytes was determined using the MicroComputer Imaging Device (Fuji Photo Film Co, Tokyo, Japan).

Assay of Plasma Free Fatty Acid Concentrations

Plasma concentrations of free fatty acid (FFA) were measured using NEFA-test Wako (Wako Pure Chemical Industries, Osaka, Japan) after extraction.

Assay of Plasma Adrenocorticotropin and Corticosterone Concentrations

Plasma concentrations of adrenocorticotropin (ACTH) and corticosterone (B) were measured by immunoradiometric assay and radioimmunoassay using the ACTH IRMA "YUKA" kit (Yuka Mdias Co, Ibaragi, Japan) and Biotrak rat corticosterone assay system (Amersham Life Science, Backinghamshire, England), respectively.

Preparation of Complementary RNA Probes

RNA probes complementary to mRNA were produced by reverse transcription polymerase chain reaction (RT-PCR) using total RNA from rat subcutaneous adipose tissue for PPAR-γ (sense primer: 5′-GGGTGAAACTCTGGGAGATTCTCC-3′ and antisense primer: 5′-TCAGCAACCATTGGGTCAGCTCT-3′) and total RNA from rat liver for rat GR (sense primer: 5′-GAGTTCAACGTCTGCAACTG-3′ and antisense primer: 5′-TCCAGGGACTCTCGTTTGTG-3′). The probe of rat GR corresponded to nucleotides 551-771. Complementary RNA probes for rat UCP1 and β-actin were produced as previously described and corresponded to nucleotides 321-536 for rat UCP1 and 3001-3120 for rat β-actin, respectively.[21] Since the probe for rat PPAR-γ includes the regions of 88 bp of the PPAR-γ2 transcript and 185 bp of the region common to both PPAR-γ1 and PPAR-γ2, 2 PPAR-γ transcripts can be detected as protected bands of either 273 bp (PPAR-γ2) or 185 bp (PPAR-γ1).[9] These probes corresponded to nucleotides 140-324 of rat PPAR-γ1 and 52-324 of rat PPAR-γ2, respectively.

The *Eco*RI-*Bam*HI fragments of PCR products of rat PPAR-γ, GR, UCP1, and β-actin were then subcloned into pGEM-3Z (Promega Corp, Madison, WI). Plasmids were linearized by the restriction enzyme *Bam*HI (Boehringer Mannheim, Mannheim, Germany). Antisense complementary (c)RNA probes were synthesized with T7 RNA polymerase (Stratagene Cloning Systems, La Jolla, CA), 100 mCi [α-^{32}P]UTP (800 Ci/mmol), and other unlabeled nucleotides using RNA transcription kits (Stratagene Cloning Systems) as previously described.[21]

RNase Protection Assay

The RNase protection assay for quantitative analysis of mRNA was performed using RPA II kits (Ambion Inc, Austin, TX) as previously described.[21] First, a 10-μg sample RNA was hybridized with 1×10^5 cpm [α-^{32}P]UTP-labeled rat PPAR-γ, GR, UCP1, or β-actin probe in 20 μL hybridization buffer (80% deionized formamide/100 mmol/L sodium citrate, pH 6.4/300 mmol/L sodium acetate, pH 6.4/1 mmol/L EDTA, Ambion) at 55°C for 16 hours. Nonannealing nucleic acids were digested with RNase A and T1 at final concentrations of 5 mg/mL and 100 U/mL, respectively, in 200 μL of RNAase digestion buffer (Ambion) at 37°C for 30 minutes. The protected fragments were electrophoresed on a 5% polyacrylamide gel (PAGE) containing 7 mol/L urea (urea-PAGE). The dried gel was exposed on an imaging plate (Fuji Photo Film Co) and the integrated optical density of the radioactive bands was quantified by an imaging analyzer (FLA 2000 and BAS 2000, Fuji Photo Film Co). The levels of PPAR-γ, GR, and UCP1 mRNA were standardized by the levels of β-actin mRNA.

Semiquantitative RT-PCR for 11β-HSD1 mRNA

11β-HSD1 mRNA expression in the omentum and subcutaneous adipose tissues was analyzed by RT-PCR using a capillary electrophoresis system with laser-induced fluorescent (LIF) detection (P/ACE system 5000, Beckman Coulter, Fullerton, CA). The RT-PCR and following PCR reaction were performed as described previously.[22] To remove contaminating genomic DNA, 15U of RNase-free DNase I (Rosche Diagnostics, Mannheim, Germany) was mixed with 5 μg of total RNA in Tris-HCl buffer containing 15 mmol/L Tris-HCl (pH 8.3), 75 mmol/L KCl, 1.5 mmol/L dNTPs, and 30 U of RNase inhibitor. The mixture was incubated at 37°C for 30 minutes, and then at 75°C for 5 minutes for inactivation of enzyme. For synthesis of the first strand of complementary (c)DNA, 7.5 U of murine leukemia virus reverse transcriptase (Roche Molecular Systems, Branchburg, NJ), 3.75 mol/L random and oligo dT primers, and 7.5 mmol/L $MgCl_2$ were then added to the solution. The mixture was incubated at 42°C for 60 minutes, 99°C for 5 minutes, and then stored at 4°C in a Gene Amp PCR system 9600 (PE Co Biosystems, Foster City, CA). The following PCR was preformed in 50 μL solution using 0.5 μL of the first-strand cDNA, 0.625 U of Taq polymerase (Takara Shuzo Co, Shiga, Japan), 10 mmol/L of Tris-HCl buffer (pH 8.3) containing 50 mmol/L KCl, 0.2 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, and 30 mmol/L primers described below. To amplify 284 bp of rat 11β-HSD1 cDNA fragment, we designed sense and antisense primers according to the nucleotide sequence 5292-5312 and 6265-6284 (sense primer: 5′-TCAGAGGCTGCTGTTGCCTG-3′ and antisense primer: 5′ CTTGCAGTCAATACCACATG-3′). Also we amplified 130 bp of rat β-actin cDNA as an internal standard using a sense and an antisense primers according to the nucleotide sequence 2309-2329 and 3111-3130 (sense primer: 5′-CCTGAGCGCAAGTACTCTGT-3′ and antisense primer: 5′-AGAAGCATTTGCGGTGACAG-3′). The PCR reaction consisted of an initial denaturing at 95°C for 4 minutes, followed by 28 cycles for 11β-HSD, 24 cycles for β-actin of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 4 minutes. The PCR for 11β-HSD and β-actin were performed in separate reaction. Each 10 μL of PCR product was mixed. This mixture was

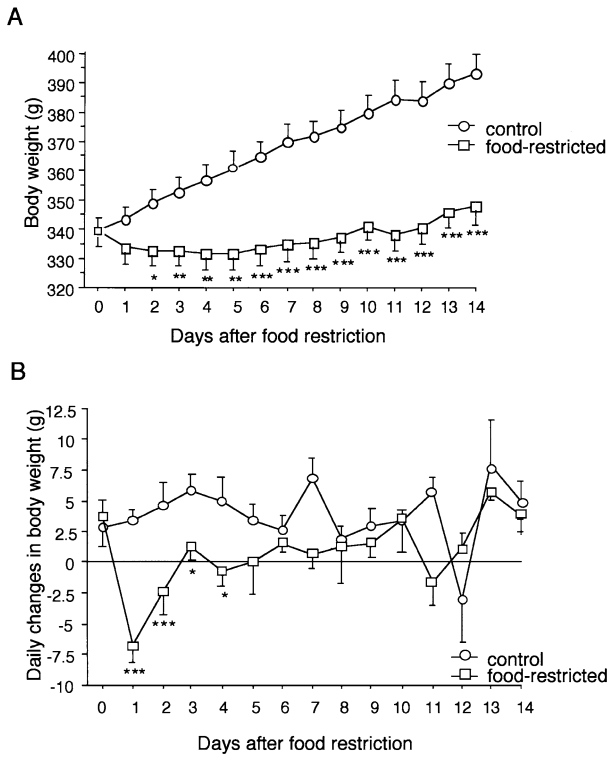

Fig 1. Body weight and daily changes in body weight during 20% food restriction. (A) Changes in body weight. Mean body weight of food-restricted rats was significantly lower than that of control rats between days 2 and 14. *$P < .05$, **$P < .01$, ***$P < .001$ *v* control rats. (B) Daily changes in body weight. The daily changes in body weight of food-restricted rats were less than those of control rats between days 1 and 4. *$P < .05$, ***$P < .001$ *v* control rats. Each group consisted of 6 rats.

separated on a capillary electrophoresis system using LIF with excitation of 488 nm and emission of 520 nm. The capillary was filled with the polymer solution consisted of 25 mmol/L MOPS-Tris (pH 7.55) containing 0.5% polyethylene oxide (molecular weight 4,000,000) and 0.4% polyethylene oxide (molecular weight 900,000) with 4 μL of EnhanCE intercalator (Beckman Coulter, Fullerton, CA) per 10 mL gel. Separations were performed at 300 V/cm for 8 minutes. To quantify the levels of the 11β-HSD1 mRNA, the area under the peak for the DNA fragment of 11β-HSD1 was normalized by that for β-actin, because the area under the peak for a particular DNA fragment can be correlated to the quantity of that fragment.[23,24] The amount of initial total RNA and number of PCR cycles were chosen so that they were in the linear range as determined by the area under the peak.

Statistical Analysis

The data of body weight and daily changes in body weight were subjected to a repeated measurement analysis of variance, (ANOVA). Other data were subjected to Mann-Whitney's *U* test and differences with $P < .05$ were considered significant. All results were expressed as the mean ± SEM.

RESULTS

Body Weight and Weight of Adipose Tissues

The mean body weight of food-restricted rats after 14 days of food restriction was approximately 13% less than that of control rats (Fig 1A). The daily increase in body weight of food-restricted rats was less than that of control rats between days 1 and 4, while there was no significant difference in daily body weight changes between the 2 groups after day 5 (Fig 1B). The weight of the omentum of food-restricted rats after 2 or 14 days was less than that of control rats, while the weight of brown, subcutaneous and epididymal adipose tissues did not differ (Table 1).

Size and Number of Adipocytes

The mean size and number of adipocytes of the omentum of food-restricted rats did not differ from those of control rats after 2 days of food restriction, while the mean size of adipocytes of the omentum after 14 days of food restriction was smaller and the mean number was greater than those of control rats (Fig 2). The mean size and number of adipocytes of the subcutaneous adipose tissue of food-restricted rats did not differ from those of control rats after 2 or 14 days of food restriction.

Plasma FFA, ACTH, and B Concentrations

Plasma FFA concentrations in food-restricted rats were higher than those of control rats after both 2 and 14 days (Fig 3). The plasma concentrations of ACTH and B in food-restricted rats did not differ form those of control rats after 2 days of food restriction, but they were higher than those of control rats after 14 days (Fig 4).

UCP1, PPAR-γ1, PPAR-γ2, GR, and 11β-HSD1 mRNA Expression in Adipose Tissues

Typical autoradiograms of PAGE produced by the RNase protection assay for PPAR-γ1, PPAR-γ2, and GR mRNA are shown in Fig 5. Figure 6 shows typical results of capillary electrophoresis with LIF detection of 11β-HSD1 and β-actin mRNA. We found that the levels of PPAR-γ2, GR, and 11β-HSD1 mRNA expression in the omentum of food-restricted rats were lower than those of control rats after 2 days, but did

Table 1. Weight of Adipose Tissues

	After 2 Days of Food Restriction		After 14 Days of Food Restriction	
	Control	Food Restriction	Control	Food Restriction
Brown adipose tissue (g)	0.406 ± 0.029	0.353 ± 0.017	0.430 ± 0.046	0.340 ± 0.042
Subcutaneous adipose tissue (g)	5.262 ± 0.318	4.875 ± 0.480	5.262 ± 0.418	5.092 ± 0.457
Omentum (g)	2.190 ± 0.067	1.790 ± 0.170*	3.228 ± 0.192	2.322 ± 0.257*
Epididymal adipose tissue (g)	2.942 ± 0.324	2.438 ± 0.269	3.873 ± 0.326	3.448 ± 0.291

*$P < .05$ *v* control.

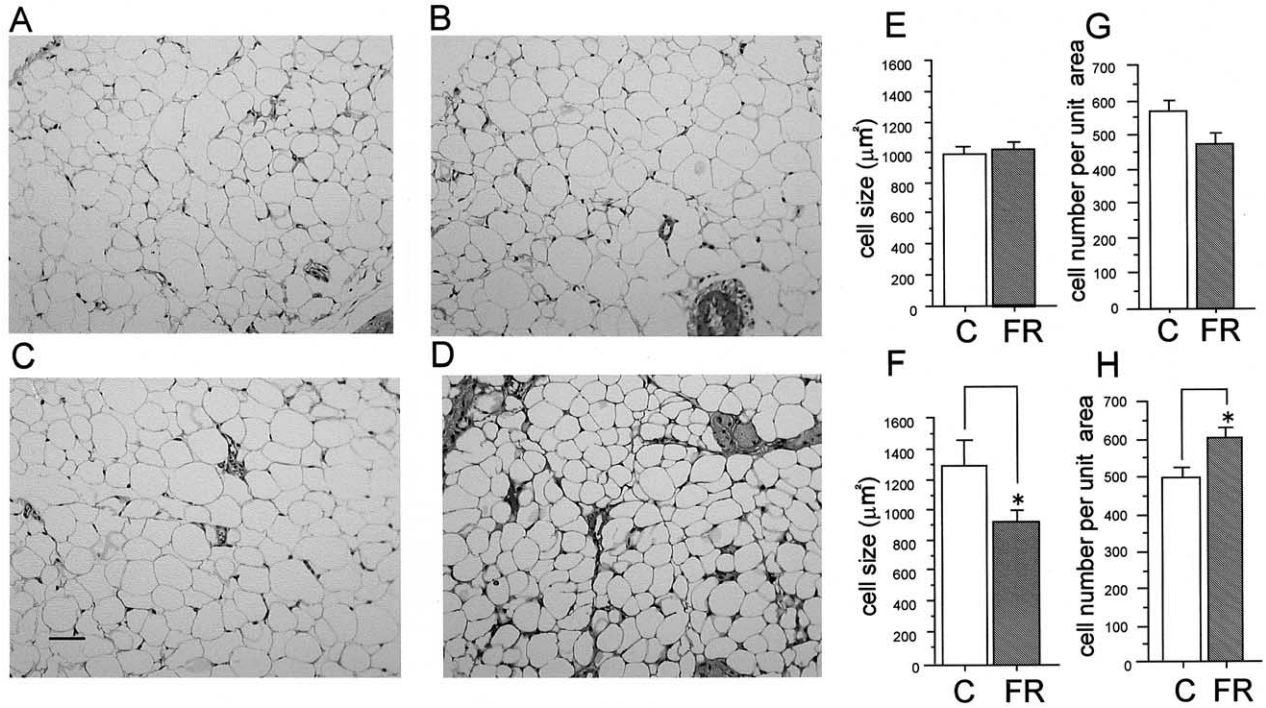

Fig 2. Hematoxylin-eosin staining of the adipocytes of the omentum. (A and B) Omentum of control (A) and food-restricted rats (B) after 2 days of food restriction. (C and D) Omentum of control (C) and food-restricted rats (D) after 14 days of food restriction. (E and F) Mean size of adipocytes of the omentum after 2 (E) or 14 days (F) of food restriction. (G and H) Number of adipocytes of the omentum after 2 (G) or 14 days (H) of food restriction. C, control; FR, food-restricted rats. *$P < .05$ *v* control rats. The black bar in the panel C indicates 50 μm. The mean size of adipocytes of the omentum of food-restricted rats did not differ from that of control rats after 2 days of food restriction, while the mean size of adipocytes of the omentum of food-restricted rats was smaller and the mean number was greater than that of control rats after 14 days of food restriction.

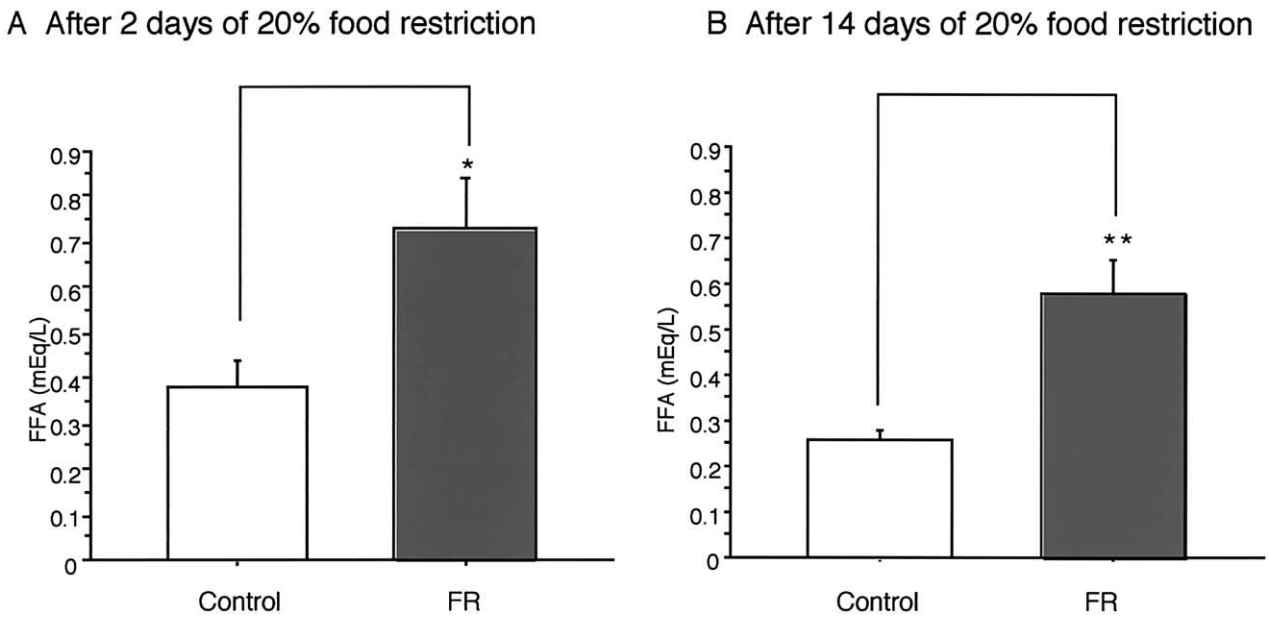

Fig 3. Plasma FFA concentrations after 2 (A) or 14 days (B) of food restriction. C, control; FR, food-restricted. *$P < .05$, **$P < .01$ *v* control rats. Plasma FFA concentrations of food-restricted rats was higher than those of control rats after both 2 and 14 days of food restriction. Each group consisted of 6 rats.

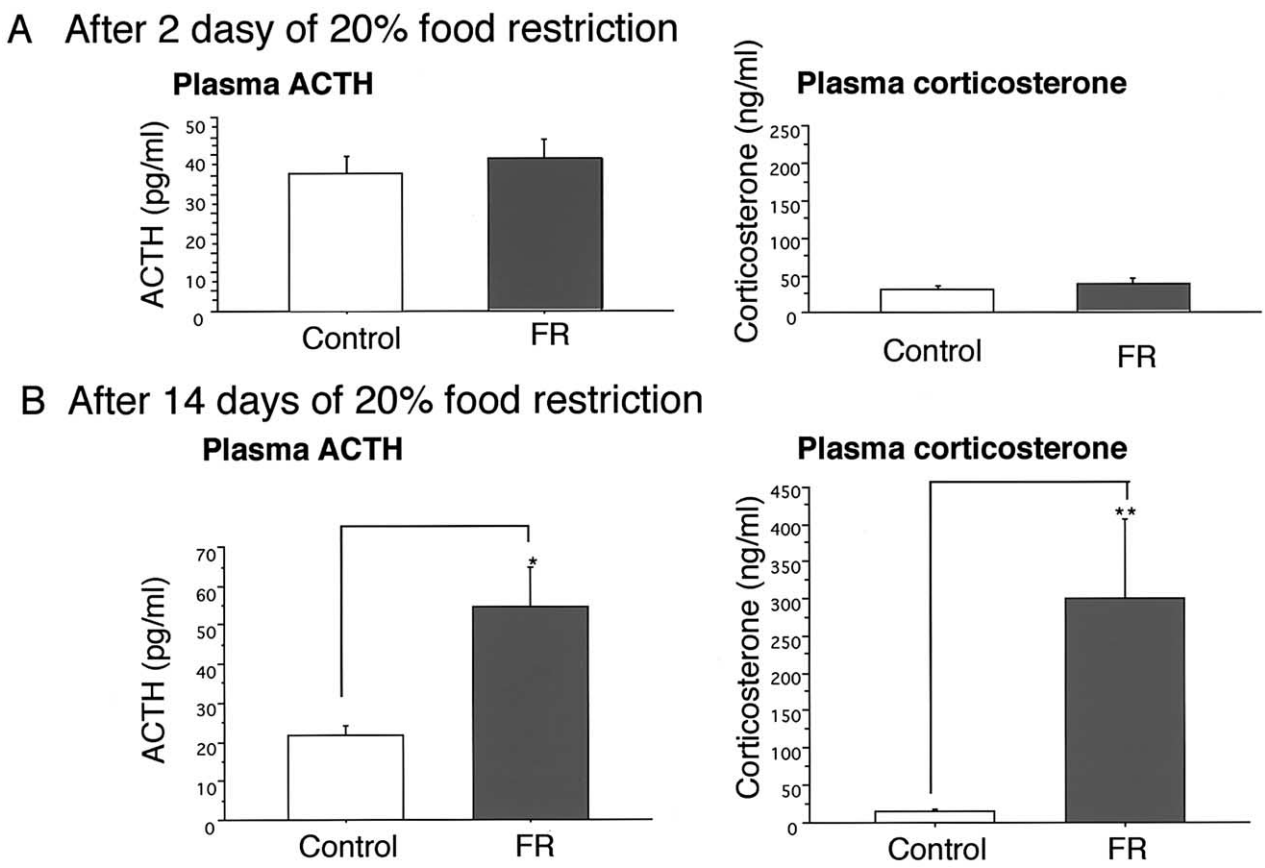

Fig 4. Plasma ACTH (left) and corticosterone (right) after (A) 2 or (B) 14 days of food restriction. C, control; FR, food-restricted rats. *$P < .05$, **$P < .01$ *v* control rats. The plasma concentrations of ACTH and B did not differ between control and food-restricted rats after 2 days of food restriction. However, the plasma concentrations of ACTH and B were higher in food-restricted rats than in control rats after 14 days. Each group consisted of 6 rats.

not differ from those of control rats after 14 days (Figs 7 and 8). Also, the levels of PPAR-γ1 mRNA expression in the omentum of food-restricted rats did not differ from those of control rats after 2 or 14 days (Fig 8). Further, the levels of PPAR-γ1, PPAR-γ2, GR, and 11β HSD1 mRNA expression in the subcutaneous adipose tissue of food-restricted rats did not differ from those of control rats after 2 or 14 days (Figs 7 and 8). Likewise, the levels of UCP1 mRNA expression in the brown adipose tissue of food-restricted rats did not differ from those of control rats after 2 or 14 days (Fig 7).

DISCUSSION

In this study, we examined the changes in the levels of PPAR-γ, GR, 11 β-HSD1 mRNA expression, body weight, and weight of adipose tissues during food restriction to help determine how these factors control adipocyte differentiation. We found that the mean size and number of adipocytes of omentum of food-restricted rats did not differ from those of control rats after 2 days, while after 14 days, the mean size was smaller and number was greater in the food-restricted rats. Also, after 2 days, the levels of PPAR-γ2 mRNA expression of food-restricted rats were lower than those of control rats, while the levels of PPAR-γ2 mRNA expression of food-restricted rats

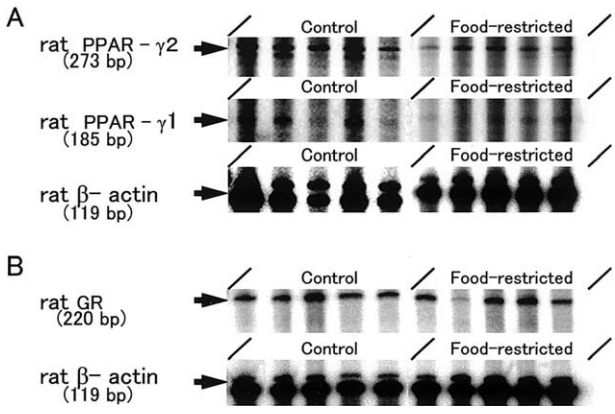

Fig 5. Typical autoradiograms of PAGE produced by the RNase protection assay. (A) Autoradiogram of PAGE produced by the RNase protection assay for PPAR-γ1, PPAR-γ2, and β-actin mRNA in the omentum of control (left 5 lanes) and food-restricted rats (right 5 lanes) after 2 days. Protected bands of 273 bp, 185 bp, and 119 bp indicate rat PPAR-γ2, PPAR-γ1, and β-actin mRNA, respectively. (B) Autoradiogram of PAGE produced by the RNase protection assay for GR and β-actin mRNA in the omentum of control (left 5 lanes) and food-restricted rats (right 5 lanes) after 2 days. Protected bands of 220 bp and 119 bp indicate rat GR and β-actin mRNA, respectively.

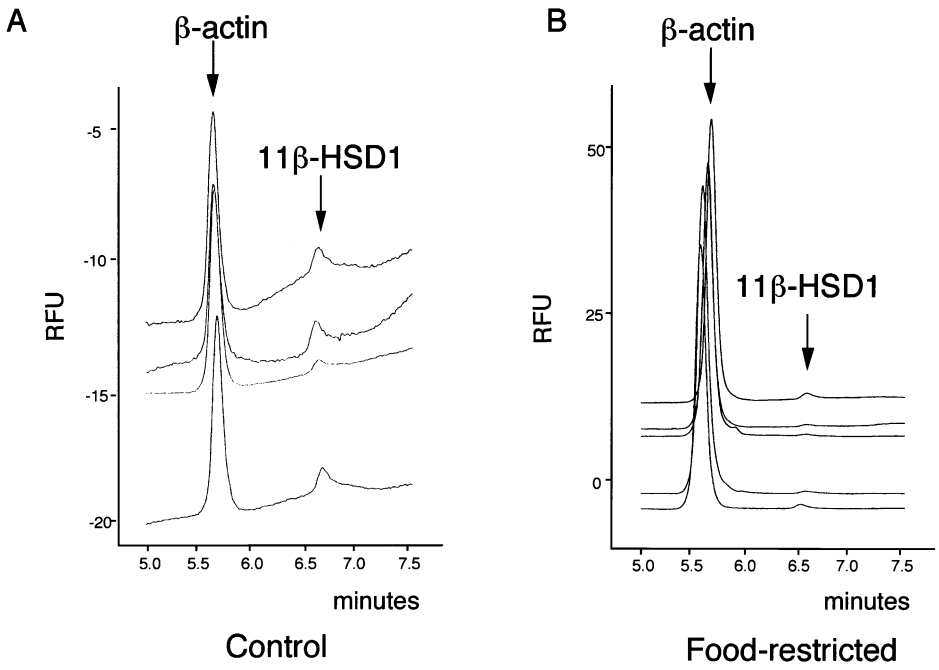

Fig 6. Typical capillary electrophoresis with LIF detection of 11β-HSD1 and β-actin of control (A) and food-restricted rats (B) after 2 days. Arrows indicate the peaks of PCR fragments of β-actin and 11β-HSD1. Migration time of the PCR fragments of β-actin and 11β-HSD1 were 5.6 and 6.6 minutes, respectively. RFU, relative fluorescence units.

returned to the levels of control rats after 14 days. Similarly, fasting has been shown to decrease the expression of PPAR-γ1 and PPAR-γ2 mRNA in the white adipose tissues of rats and mice[8,9] and a low calorie diet reduces PPAR-γ2 mRNA levels in obese humans.[12] Therefore, it seems that the decrease in PPAR-γ2 mRNA levels observed in the omentum of rats which were food-restricted for 2 days was induced by the reduced energy intake. PPAR-γ is known to induce the differentiation of 3T3-L1 cells.[13] In addition, activation of PPAR-γ by TZDs induces the differentiation of adipocytes in normal rats.[11] In general, the levels of PPAR-γ mRNA expression reflect the degree of adipocyte differentiation.[25] Given this background, the decrease in the PPAR-γ2 mRNA levels observed in the omentum of food-restricted rats after 2 days may reflect a decrease in adipocyte differentiation. In contrast, after 14 days of food restriction, there was an increase in the number of small adipocytes in the omentum. It seems that an increase in the number of small newly differentiated adipocytes contributed to the decrease in size and the increase in the number of omentum adipocytes in the food-restricted rats because the recovery of the PPAR-γ2 mRNA expression levels in the adipocytes was found. In addition, it is also likely that the decrease in size of omentum adipocytes in the food-restricted rats might be due to the lipolysis because plasma concentrations of FFA were increased by food restriction.

The concentration of GC in the vicinity of GR and the number and affinity of GR define the action of GC. The local concentrations of GC are affected by the activity and amount of 11β-HSD1 because this enzyme changes inert GC (11-dehydrocorticosterone or cortisone) to activated form (corticosterone or cortisol).[14] We found that, in parallel with the levels of PPAR-γ2 mRNA expression, the levels of GR and 11β-HSD1 mRNA expression were lower in the omentum after 2 days in the food-restricted rats, while they did not differ between the food-restricted and control rats after 14 days. GC induces PPAR-γ1 and PPAR-γ2 mRNA expression and activates the differentiation of the isolated human adipocytes and 3T3-L1 cell.[12] Transgenic mice overexpressing 11β-HSD1 selectively in adipose tissue show increased levels of B in adipose tissue and visceral obesity.[26] Therefore, although the plasma concentrations of B did not decrease after 2 days of food restriction, the decreased levels of GR and 11β-HSD1 mRNA expression in the omentum could suppress the levels of PPAR-γ2 mRNA expression by attenuating the local GC effects.

It is possible that low levels of PPAR-γ2 mRNA expression found after 2 days of food restriction enhanced 11β-HSD1 mRNA expression because PPAR-γ agonists are known to inhibit the levels of 11β-HSD1 mRNA expression.[27] In addition, the recovery of the levels of PPAR-γ2 mRNA expression in the omentum after 14 days of food restriction may be due to increased local GC effects; although the levels of GR and 11β-HSD1 mRNA in the omentum returned to the levels of control rats after 14 days, the plasma concentrations of B were elevated, suggesting that there were enhanced GC effects in the omentum.

Low levels of 11β-HSD1 mRNA expression in the omentum were also found after 2 days of food restriction. This may be linked to the decrease in the omentum mass of food-restricted rats through cytokines or leptin produced by adipose tissues. For example, plasma concentrations of leptin positively correlate with body mass index (BMI) and percent body fat in rodents and humans, and decrease when BMI is reduced by calorie restriction in humans.[28] In addition, plasma concentrations of tumor necrosis factor-α (TNF-α) positively correlated with BMI or intra-abdominal fat mass in humans.[29] Also, cytokines, such as TNF-α and interleukin-1β (IL-1β), and leptin have been reported to increase the expression of 11β-HSD1 mRNA in adipose tissues.[30] Therefore, the decreases in

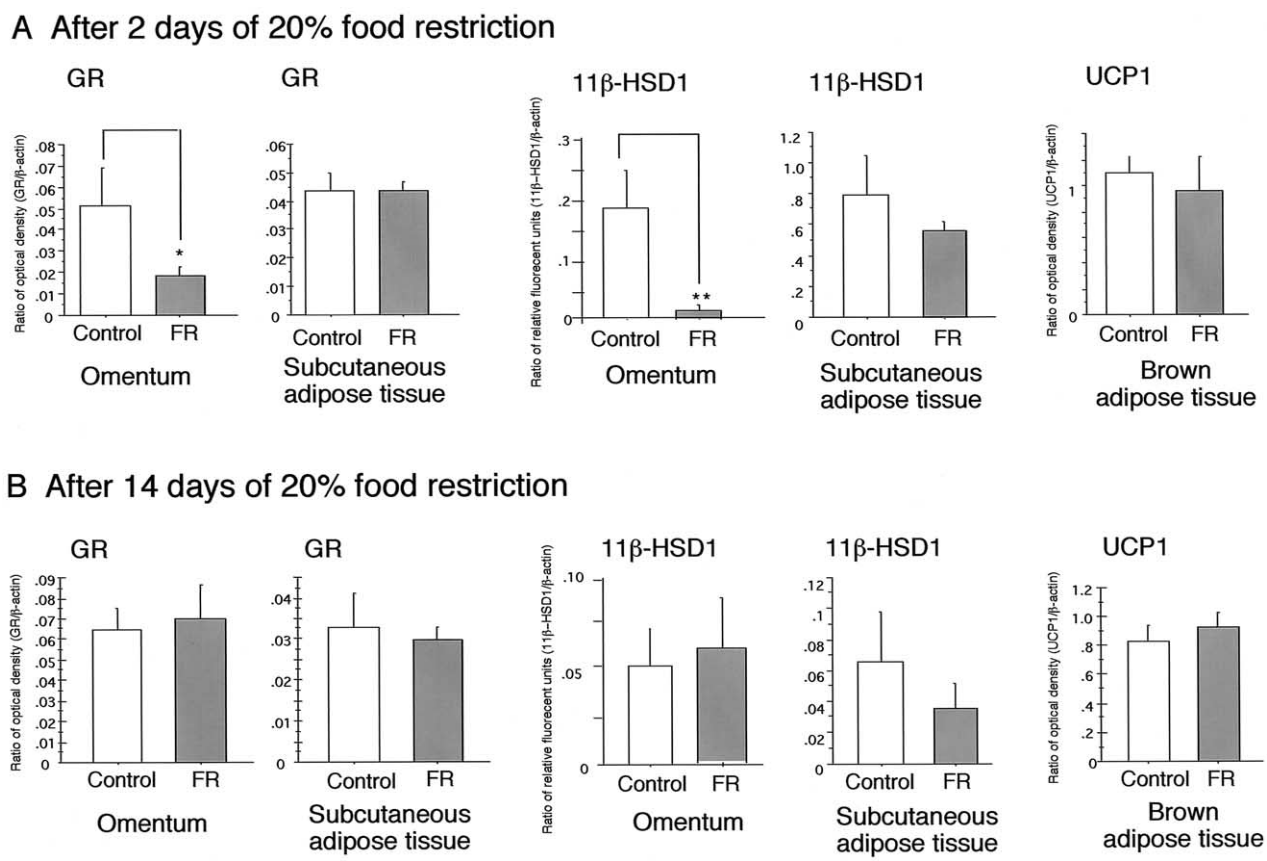

Fig 7. GR and 11β-HSD1 mRNA expression in the omentum and subcutaneous adipose tissue and UCP1 mRNA expression in brown adipose tissue after (A) 2 or (B) 14 days of food restriction. Levels of GR and 11β-HSD1 mRNA expression in the omentum of food-restricted rats after 2 days of food restriction were lower than those of control rats. Levels of UCP1 mRNA expression in the brown adipose tissue of food restricted rat after 2 or 14 days did not differ from those of control rats. C, control; FR, food-restricted rats. **$P < .01$ *v* control rats. Each group consisted of 6 rats.

cytokines and leptin levels may be involved in the mechanism underlying the decrease in 11β-HSD1 mRNA expression levels found after 2 days of food restriction, although the plasma levels of cytokines and leptin were not measured.

In contrast to the omentum, the weight and mean size of the adipocytes and levels of PPAR-γ2, GR, and 11β-HSD1 mRNA expression in the subcutaneous adipose tissue did not differ between the control and food-restricted rats after 2 or 14 days. TZD-stimulated preadipocyte differentiation is much greater in subcutaneous than visceral preadipocytes.[31] However, both the reductase activity of 11β-HSD1 and GR density are reported to be higher in the omentum than in the subcutaneous adipose tissue.[32,33] Thus, local GC effects may be enhanced more efficiently in the omentum compared to the subcutaneous adipose tissue resulting in the changes in PPAR-γ mRNA expression levels of the omentum as shown in this study.

Similar to our findings in the present study, a 10% reduction in body weight in obese people decreases the levels of PPAR-γ2 mRNA expression in abdominal adipose tissue, but the levels of PPAR-γ2 mRNA expression return to pretreatment levels after 4 weeks of maintenance of reduced body weight.[12] This phenomenon may be an adaptation for reduced energy intake. In fact, the immature small adipocytes take up much more glucose than large adipocytes at submaximal levels of insulin and the smaller adipocytes are more sensitive to the antilipolytic action of insulin.[34,35] Thus, an increased population of small adipocytes may be beneficial for survival under conditions of reduced energy intake, because they more sufficiently take glucose and store triglyceride. However, if this adaptation occurs during sustained low-calorie diet, it may result in the restorage of the lipid in the adipose tissue and may attenuate weight loss or induce a regain of body weight.

Decreased energy intake during fasting or approximately 60% to 70% food restrictions induces a reduction of UCP1 mRNA in the brown adipose tissue of rats.[18,19] However, we did not observe a change in the levels of UCP1 mRNA expression in the brown adipose tissue of food-restricted rats. This suggests that the 2 or 14 days with a 20% food restriction did not suppress energy expenditure by nonshivering thermogenesis. A 20% food restriction is a mild reduction of energy intake compared to fasting or approximately 60% to 70% food restrictions because the food-restricted rats gained weight over a 14-day period of food restriction after a brief period of adaptation.

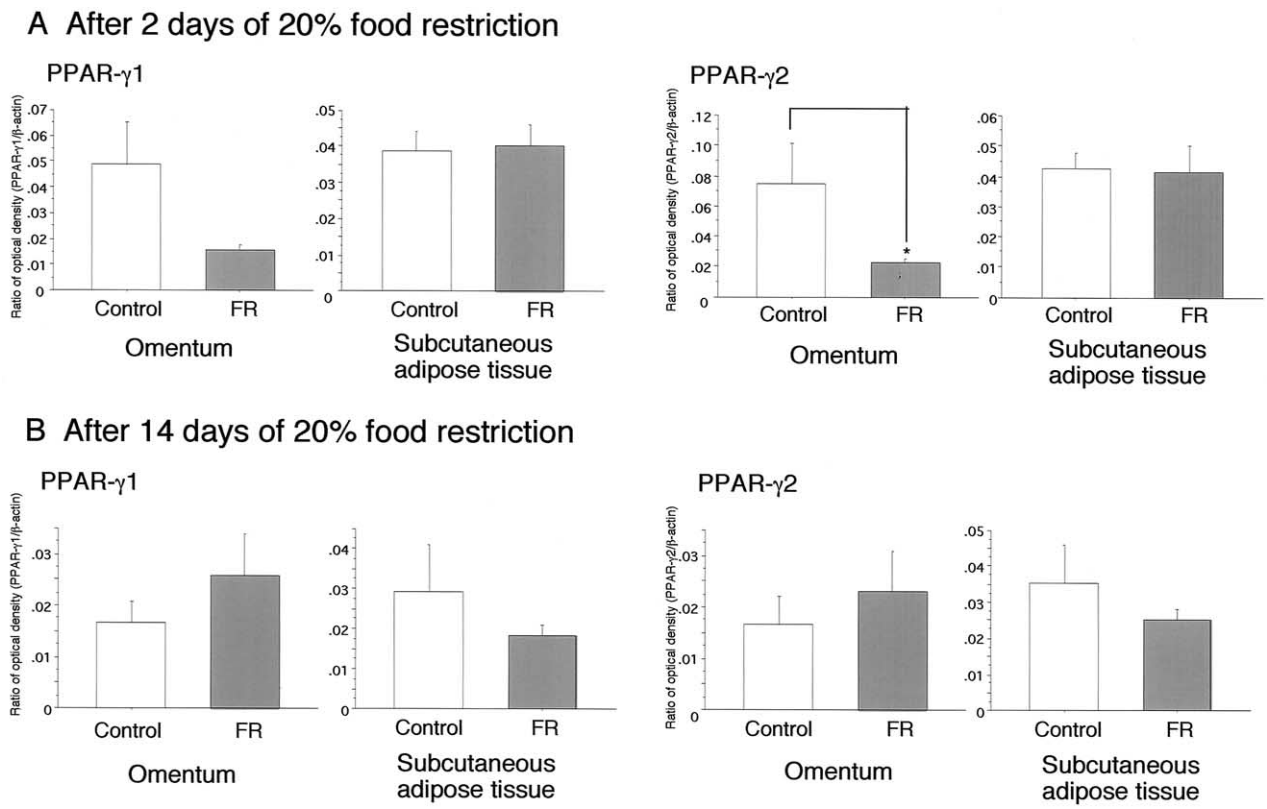

Fig 8. PPAR-γ1 and PPAR-γ2 mRNA expression in the omentum and subcutaneous adipose tissue after (A) 2 or (B) 14 days of food restriction. Levels of PPAR-γ2 mRNA expression in the omentum of food-restricted rats after 2 days were lower than those of control rats. C, control; FR, food-restricted rats. *$P < .05$ *v* control rats. Each group consisted of 6 rats.

In conclusion, our results suggested that there is a transient attenuation of adipocyte differentiation in the omentum after 2 days of a 20% reduction in food intake. Adipocyte differentiation appears to recover after 14 days of food restriction, resulting in an increased population of small adipocytes. Our results also indicate that food restriction alters the efficiency of local GC effects in the adipose tissue in a time course-dependent manner by altering GR and 11β-HSD1 mRNA expression. This may work in combination with the higher levels of plasma GC induced by food restriction to induce the recovery of the levels of PPAR-γ2 mRNA expression in the omentum, thereby promoting the recovery of adipocytes differentiation.

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