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Exercise before or after refeeding prevents refeeding-induced recovery of cell size after fasting with a different pattern of metabolic gene expressions in rat epididymal adipocytes

Takuya Sakurai[a,b,1], Megumi Takei[a,1], Junetsu Ogasawara[a], Hiroshi Ueda[a], Takako Kizaki[b], Hideki Ohno[b], Tetsuya Izawa[a,c,*]

[a]*Department of Kinesiology, Graduate School of Science, Tokyo Metropolitan University, Tokyo 192-0397, Japan*
[b]*Department of Molecular Predictive Medicine and Sport Science, School of Medicine, Kyorin University, Tokyo 181-8611, Japan*
[c]*Health Promotion Science Group, Graduate School of Human Health Science, Tokyo Metropolitan University, Tokyo 192-0397, Japan*

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Abstract

We investigated the effect of exercise before or after refeeding on cell size and on the expression of several messenger RNAs (mRNAs) involved in lipolysis and lipogenesis in fasted rat epididymal adipocytes. Fasting for 65 hours reduced the diameter of adipocytes to 72.0 μm from 78.4 μm in fed control rats, whereas refeeding for 1 or 2 days restored adipocyte size to 74.0 or 75.8 μm, respectively. Exercise before or after refeeding blocked refeeding-induced restoration of adipocyte size and led to adipocyte size similar to that observed after fasting. Fasting dramatically reduced expression of the fatty acid synthase mRNA, although expression of this gene returned to the control level after refeeding. However, exercise after but not before refeeding inhibited recovery of the expression of fatty acid synthase mRNA resulting from refeeding. In contrast, exercise before but not after refeeding led to enhanced expression of mRNAs encoding the hormone-sensitive lipase and β_3-aderenoceptor. Thus, exercise before or after refeeding prevents refeeding-induced restoration of adipocyte size after fasting via different pathways. Exercise before and after refeeding enhanced the expression of lipolytic mRNAs or inhibited the expression of lipogenic mRNAs, respectively.

1. Introduction

The lipolytic and lipogenic responses of adipocytes are quite sensitive to dietary manipulation. For example, short-term fasting enhances adipocyte lipolysis and hormone-sensitive lipase (HSL) activity [1,2] and increases expression of the messenger RNAs (mRNAs) encoding HSL and β_3-adrenergic receptor (β_3-AR) [2-4], whereas severe fasting leads to reduced adipocyte lipolytic responses but paradoxically enhanced the amount of [^{3}H]dihydroalprenolol, a β-antagonist, specifically bound in rat adipocyte membranes [5]. Moreover, the expression of mRNAs encoding various lipogenic enzymes is also down-regulated in mouse adipocytes after 7.5 hours of fasting, but refeeding after fasting increases the expression of these mRNAs beyond their basal levels [6]. Furthermore, repeated cycles of fasting and refeeding increase the expression of fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase (ACC) mRNAs significantly [7] but reduces the expression of β_3-AR mRNA [4]. Thus, dietary manipulation alters the ability of adipocytes to undergo lipolysis and lipogenesis due to changes in the expression of genes controlling these metabolic pathways in adipocytes, and refeeding after fasting appears to increase the expression of lipogenic genes. As a result, adipose tissue remodeling can occur, leading to a rapid and excessive increase in the size and mass of both adipocytes and adipose tissue during the fasting-refeeding cycle. Therefore, it is important to identify ways to prevent such increases in the size and mass of adipocytes to prevent repeated bouts of weight loss and regain, which are

* Corresponding author. Health Promotion Science Group, Graduate School of Human Health Science, Tokyo Metropolitan University, Tokyo 192-0397, Japan. Tel.: +81 426 77 2962; fax: +81 426 77 2961.
E-mail address: izawa@comp.metro-u.ac.jp (T. Izawa).
[1] These authors contributed equally to this work.

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often induced by the cognitive restraint of nutritional intake-induced weight loss, a phenomenon known as weight cycling or "yo-yo" dieting.

In this respect, Griffiths et al [8,9] have reported interesting findings in rat liver, indicating that exercise can prevent refeeding-induced increases in FAS mRNA expression. This finding suggests that exercise is important as a tool to prevent weight cycling and to control body weight. However, there is little information on the effect of exercise on dietary manipulation–induced changes in lipolytic and lipogenic genes in adipocytes themselves, and no direct evidence has so far been obtained. Clarifying these issues will help strengthen our understanding of the role of exercise in controlling body weight and, subsequently, to prevent disorders associated with obesity.

In the present study, we investigated the effects of exercise before or after refeeding after fasting on cell size and the expression of the following genes, which are involved in lipolysis and lipogenesis in rat epididymal adipocytes: HSL, β_3-AR, adipose lipid-binding protein (ALBP), sterol regulatory element-binding protein (SREBP)-1c, FAS, ACC, acetyl-coenzyme A synthetase (ACS), lipoprotein lipase (LPL), and peroxisome proliferator activator receptors (PPAR)-γ1 and -γ2. The data show that exercise before and after refeeding can prevent a refeeding-induced increase in cell size after fasting. However, exercise before refeeding enhanced the expression of genes involved in lipolytic pathways, such as β_3-AR and HSL, whereas exercise after refeeding lowered the expression of lipogenic enzymes, such as FAS, ACC, and ACS.

2. Materials and methods

2.1. Animal care and exercise training program

Male Wistar rats (Japan SLC, Hamamatsu, Japan) with an initial body weight of approximately 350 g each were housed in a cage in a temperature-controlled room at 23°C with a 12-hour (6:00 AM-6:00 PM) light-dark cycle. All animals were fed the same diet (standard rodent chow MF; Oriental Yeast Ltd, Tokyo, Japan) except during fasting but had ad libitum access to water. The animals were randomly assigned to 6 experimental groups (Fig. 1): sedentary control rats that performed no manipulation (C; n = 7); rats fasted for 65 hours (F; n = 7); rats refed for 24 hours (RF1; n = 7) or 48 hours after 65 hours of fasting (RF2; n = 9); rats exercised before refeeding after 65 hours of fasting and then killed 24 hours after refeeding (EX-RF; n = 7); rats exercised after 24-hour refeeding after 65 hours of fasting, and then there was a second 24-hour refeeding period and thereafter the rats were killed (RF-EX; n = 9). For fasting experiments, food was removed at 5:00 PM, and after a 65-hour fasting period, food was then supplied at 10:00 AM. The rats of the EX-RF and RF-EX groups performed treadmill running at an estimated work rate of more than 50% maximal oxygen uptake (set at a 5° incline for 60 minutes at 17 m/min) at

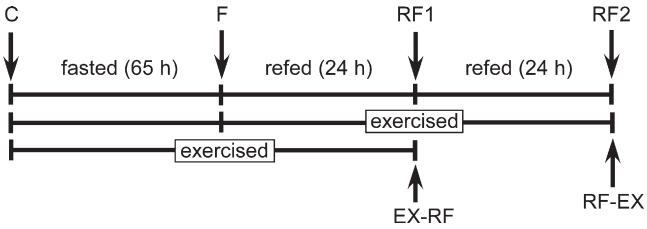

Fig. 1. Experimental protocol. The animals were randomly divided into 6 groups: sedentary controls (C), fasting for 65 hours (F), refeeding for 24 or 48 hours after fasting (RF1 and RF2, respectively), exercising before refeeding after fasting (EX-RF), and exercising post refeeding after fasting (RF-EX). The arrows indicate the sampling point of each group.

9:00 AM, before refeeding or 1 day after refeeding, respectively. This intensity of exercise was estimated using published data [10,11]. Before beginning the formal experiments, the animals were habituated to treadmill running (<10 m/min, <20 min/d) for 2 consecutive days; and 2 days after the habituation, they began fasting. The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100g body weight; Abbott, Abbott Park, IL) at 10:00 AM, and the body weight was then obtained, along with blood samples and fat pads. All experiments conducted in this study were approved by the Animal Care Committee of the Tokyo Metropolitan University Graduate School of Science (Tokyo, Japan).

2.2. Preparation of adipocytes

Adipocytes were isolated using a modification of the method of Rodbell [12]. Fat pads were minced with scissors and placed in plastic vials in a buffer (Krebs-Ringer bicarbonate solution buffered with 10 mmol/L HEPES, pH 7.4, containing 5.5 mmol/L glucose and 2% [wt/vol] fatty acid–free bovine serum albumin) with 200 nmol/L adenosine and collagenase type 1 (1 mg/mL, Worthington Biochemical, Freehold, NJ). Collagenase digestion was performed at 37°C in a water bath shaker. After 20 minutes, the contents of the vials were immediately filtered and centrifuged for 1 minute at 100*g*. The layer of floating cells was then washed 3 times with the buffer without collagenase.

2.3. Reverse transcriptase–polymerase chain reaction analysis

Total RNA was prepared from approximately 1×10^8 adipocytes using ISOGEN (NIPPON GENE, Tokyo, Japan). First-strand complementary DNA was obtained by incubating total RNA samples (2 μg) with reverse transcriptase (Superscript II, GIBCO BRL, Gaithersburg, MD) in the reaction mixture (18 μL). The reverse transcription product (1 μL) was subjected to polymerase chain reaction (PCR) using *Taq* DNA polymerase (Perkin Elmer, Branchburg, NJ). Twenty to thirty cycles of amplification were carried out using the following conditions for each cycle: denaturing at 94°C for 1 minute, annealing at 72°C for 2 minutes, and extension at 72°C for 3 minutes. The PCR products were electrophoresed

in 1% agarose gels containing ethidium bromide. The intensity of the bands was estimated by scanning with a Light-Capture Scanner (ATTO, Tokyo, Japan), and the optical density of each band was analyzed with the CS Analyzer (ATTO). The primers used are described as follows:

HSL 5′-CATAAGACCCCATTGCCTGC-3′,
5′-TCTACCACTTTCAGCGTCAC-3′
β_3-AR 5′-TGTTCGTCGTAGCTAAGCGC-3′,
5′-CTTCATAGCCATCAAACCTG-3′
ALBP 5′-CTTACAAAATGTGCGACGCC-3′,
5′-ACTCTTGTAGAAGTCACGCC-3′
SREBP-1c 5′-TGAACTGTATCGCTCAGCCC-3′,
5′-GGCCACAAGAAGTAGATCAC-3′
FAS 5′-TGGACCTCATCACTAGAAGC-3′,
5′-ACATGTGATGGTCCTGTTTG-3′
ACC 5′-ATACCTGTGGGAGTAGTTGC-3′,
5′-TCCTTCAACTTGCTCTCCAG-3′
ACS 5′-TCACCACTCACATGCAGTAC-3′,
5′-CACGTATACCAACTGCCTAG-3′
LPL 5′-ACCATCAGGATATAGCACCC-3′,
5′-CGAAATGCTAACGATTGGGC-3′
PPAR-γ1 5′-ACAAGGACTACCCTTTACTG-3′,
5′-GATGGCATTGTGAGACATCC-3′
PPAR-γ2: 5′-ACTGCCTATGAGCACTTCAC-3′,
5′-GATGGCATTGTGAGACATCC-3′
18S ribosomal RNA 5′-ATAATGGAATAGGACCG-CGG-3′, 5′-GAGAGTCTCGTT-CGTTATCG-3′

2.4. Blood samples and analysis

Blood samples were collected from the abdominal aorta, and plasma was separated with a centrifuge at 3000 rpm for 15 minutes at 4°C. Samples were stored at −80°C until measurement. Insulin (insulin enzyme immunoassay kit; Morinaga Biochemical, Yokohama, Japan) and leptin (YK050 rat leptin enzyme-linked immunosorbent assay kit; Yanaihara Institute, Fujinomiya, Japan) were measured in plasma in duplicate using the commercially available kits indicated in parenthesis.

2.5. Measurement of the diameter of adipocytes

According to the modification method [13] of de Souza et al [14], intact adipocyte size distributions were determined on freshly isolated adipocytes using a Coulter counter equipped with a 400-μm orifice tube, a stirred sample chamber, and a multichannel particle analyzer (Multisizer-3 COULTER COUNTER; Beckman Coulter, Fullerton, CA). Adipocytes were diluted in a 25-mL electrolyte solution (Isoton II containing 10% glycerol) and stirred to maintain an even suspension at 37°C. Cell number and size profiles were generated using the siphon option with the manometer set for 2 mL, and adipocyte diameters were distributed across 256 channels. Using the Accucomp software supplied with the Multisizer-3, we performed analyses of adipocyte size distributions on cells ranging from 30 to 200 μm in diameter, where each channel was expressed as a percentage of the total number of cells within a given size range.

2.6. Data and statistical method

Values represent means ± SE. The significance of the difference between the means was assessed by the *t* test or the Scheffé test after significant differences among groups were established by analysis of variance. A least squares linear regression analysis was used to determine the relationship between the indicated parameters. Statistical significance was accepted at $P < .05$.

3. Results

3.1. Effects of short-duration exercise on body weight and food intake in rats subjected to a fasting-refeeding cycle

Several characteristics of the rats are depicted in Table 1. A 65-hour fast induced an approximately 40-g reduction in total body weight. The weight of fat pads was also reduced by

Table 1
Body weight, food intake, adipose tissue weight, and adipocyte diameter

	C (n = 7)	F (n = 7)	RF1 (n = 7)	EX-RF (n = 7)	RF2 (n = 9)	RF-EX (n = 9)
Body weight *						
Weight (g)	369.8 ± 3.3	330.9 ± 9.6[a]	349.3 ± 25.0	345.6 ± 28.8	354.5 ± 36.3	350.5 ± 19.1
Body mass gain after refeeding						
Absolute gain (g)			18.4 ± 1.5	14.6 ± 1.3	23.5 ± 2.4	19.6 ± 1.1
Relative gain (%)			5.3 ± 0.4	4.2 ± 0.4	6.6 ± 0.6	5.6 ± 0.3
Food intake						
g/kg of body weight per day	48.7 ± 1.4	–	64.9 ± 1.0[a]	55.3 ± 2.8[c]	49.9 ± 2.2[c]	61.1 ± 2.1[a,d,e]
Epididymal adipose tissue weight (g)						
Weight (g)	4.43 ± 0.10	3.59 ± 0.29[a]	3.84 ± 0.1[a]	3.62 ± 0.29[a]	4.01 ± 0.64	3.78 ± 0.29[a]
Tissue/body weight (%)	1.12 ± 0.02	1.08 ± 0.09	1.10 ± 0.04	1.05 ± 0.08	1.13 ± 0.03	1.08 ± 0.06
Mean fat cell size (diameter, μm)						
30-50	38.5 ± 0.1	39.1 ± 0.1	38.7 ± 0.2	38.8 ± 0.1	39.0 ± 0.3	38.9 ± 0.3
>50	78.4 ± 0.4	72.0 ± 0.7[a]	74.0 ± 0.4[a]	72.7 ± 0.4[a,c]	75.8 ± 0.5[a,b]	71.2 ± 0.3[a,d]

Values are expressed as mean ± SE. The abbreviations are described in the legend to Fig. 1. $P > .05$ or less: a, vs C; b, vs F; c, vs RF1; d, vs RF2; e, vs EX-RF.
* Value at sampling time (see Fig. 1).

fasting. The weight gain of fed control rats was 3 g per day (results not shown), but refeeding of fasted rats led to a weight gain of approximately 18 g during the first 24 hours (RF1) and a further gain of approximately 5 g during an additional 24 hours of refeeding (RF2). Neither the absolute nor the relative weight gain after refeeding was statistically significant in exercised groups (EX-RF and RF-EX rats) compared with each control group (RF1 or RF2 rats, respectively). The food intake of RF1 rats was significantly greater than that of C rats, whereas the food intake of RF2 rats during the second day of refeeding was comparable to the daily intake of C rats. However, EX-RF rats consumed less food than RF1 rats, whereas RF-EX rats consumed more food than RF2 rats.

3.2. Changes in adipocyte size

We determined the cell size distribution profiles of freshly isolated adipocytes using a Coulter counter. The instrument provides a unique methodology for the assessment of cell size by sorting aliquots of freshly isolated adipocytes according to their size over a range of 30 to 200 μm in diameter. Fat cell size was largely distributed in 2 populations, those in a 30- to 50-μm diameter range and those greater than 50 μm in diameter. We thus subsequently determined the mean fat cell size in the populations of cells larger than or smaller than 50 μm in diameter (Table 1). Neither dietary manipulation alone nor its combination with exercise altered the size distribution of adipocytes smaller than 50 μm. However, in fat cells larger than 50 μm, both treatments induced a significant change in the size distribution of adipocytes. The mean diameter of adipocytes in C rats was greater than that observed in the other groups. A 65-hour fast reduced the mean diameter of adipocytes to 72.0 μm, whereas 1 or 2 days of refeeding restored adipocyte size to 74.0 (RF1 rats) or 75.8 μm (RF2 rats), respectively. Interestingly, exercise either before or after refeeding prevented the refeeding-induced restoration of adipocyte size and maintained a cell size similar to that observed after fasting: the mean adipocyte size of EX-RF and RF-EX rats was smaller than that of RF1 and RF2 rats, respectively.

3.3. Effect of exercise on the expression of lipolytic and lipogenic genes after refeeding

One of the factors that determine adipocyte size is the balance between lipolysis and lipogenesis. Therefore, we next examined the effect of dietary manipulation and its combination with exercise on the expression of lipolytic (HSL, β_3-AR, and ALBP) (Fig. 2) and lipogenic (FAS, ACC, ACS, LPL, SREBP-1c, PPAR-γ1, and PPAR-γ2) mRNAs (Fig. 3) in adipocytes using reverse transcriptase–PCR analysis. The expression of β_3-AR mRNA was significantly reduced by fasting, and the reduced expression was maintained after refeeding (RF1 and RF2 rats). On the other hand, exercise before refeeding (EX-RF rats), but not after refeeding (RF-EX rats), enhanced the expression of HSL and β_3-AR mRNA to a greater extent than it did in F, RF1, RF2, and RF-EX rats. The expression of ALBP mRNA was not markedly changed in any of the groups.

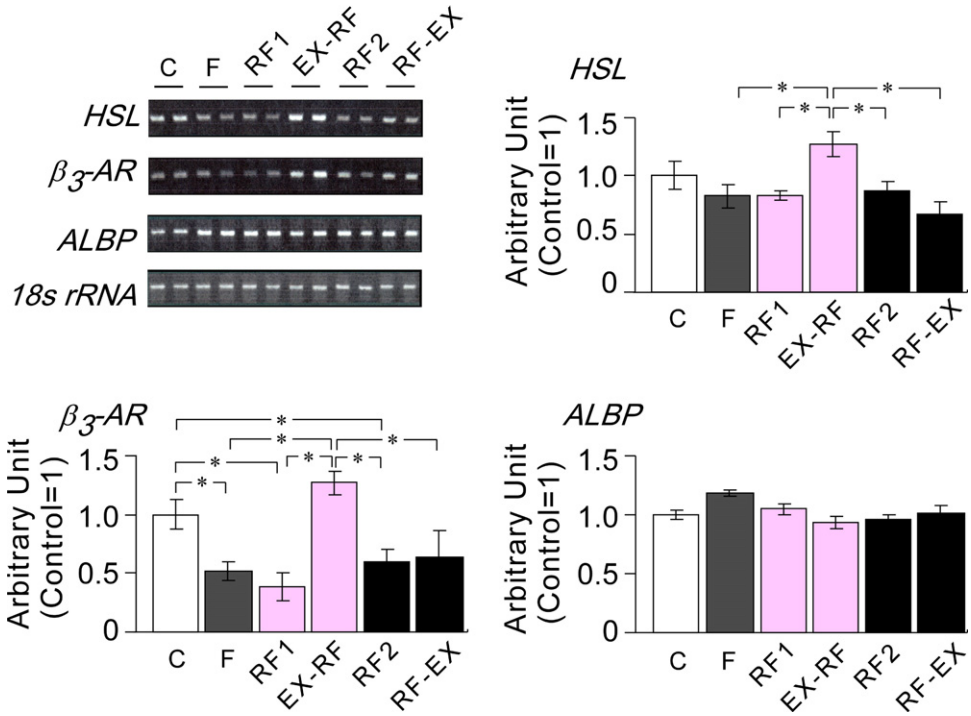

Fig. 2. Expression of lipolytic genes in adipocytes. Representative data of HSL, β_3-AR, and ALBP mRNA are shown in rat adipocytes from each experimental condition (see legend to Fig. 1). The value is related to the optical density of the C rats (sets to equal 1), and the mean ± SE is given (n = 3-5). *$P < .05$.

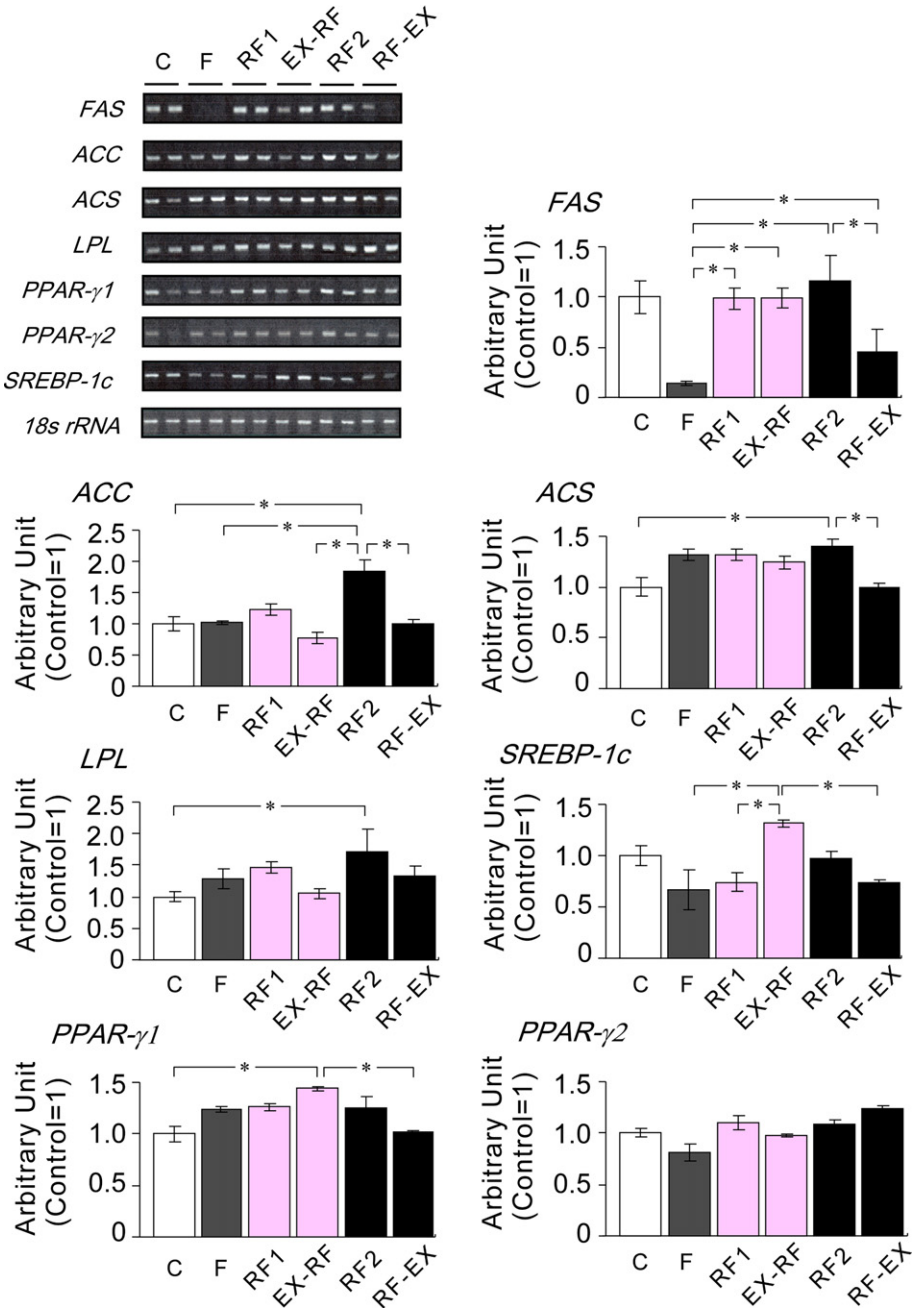

Fig. 3. Expression of lipogenic genes in adipocytes. Representative data of SREBR-1c, FAS, ACC, ACS, LPL, PPAR-γ1, and PPAR-γ2 mRNA are shown in rat adipocytes from each experimental condition (see legend to Fig. 1). The value is related to the optical density of the C rats (sets to equal 1), and the mean ± SE is given (n = 3-5). *$P < .05$.

Fasting reduced the expression of FAS mRNA significantly (Fig. 3). Fatty acid synthase mRNA expression in RF1, EX-RF, and RF2 groups returned to a level comparable to that in C rats. However, exercise after refeeding inhibited the refeeding-induced recovery of FAS mRNA expression: the expression of FAS mRNA was lower in RF-EX rats than in RF2 rats. A similar pattern of expression was observed for ACC and ACS. Greater expression of both ACC and ACS mRNA was observed 2 days after refeeding (RF2 rats), but exercise after refeeding inhibited refeeding-induced increases in ACC and ACS mRNA expression: the expression of both genes was lower in RF-EX rats than in RF2 rats. On the other hand, the expression of LPL mRNA was also higher in RF2 rats than in C rats, but exercise had no significant effect on the 2-day refeeding-induced enhancement of LPL mRNA expression.

We next investigated the expression of SREBP-1c and PPAR mRNA (Fig. 3). These encode important transcription factors that regulate the expression of lipogenic mRNAs in adipocytes. Indeed, 3 types of SREBP (SREBP-1a, SREBP-

Table 2
Plasma levels of insulin and leptin

ng/mL	C (n = 7)	F (n = 7)	RF1 (n = 7)	EX-RF (n = 7)	RF2 (n = 9)	RF-EX (n = 9)
Insulin	5.22 ± 0.75	1.38 ± 0.35[a]	4.68 ± 0.99[b]	4.28 ± 0.30[b]	5.36 ± 1.16[b]	3.37 ± 0.13[a,b,c,d,e]
Leptin	4.10 ± 0.62	0.34 ± 0.05[a]	2.09 ± 0.09[a,b]	2.25 ± 0.10[a,b]	3.24 ± 0.20[b,c]	2.41 ± 0.12[a,b,d]

Values are expressed as mean ± SE. The abbreviations are described in the legend to Fig. 1. $P > .05$ or less: a, vs C; b, vs F; c, vs RF1; d, vs RF2; e, vs EX-RF.

1c, and SREBP-2) mediate the expression of enzymes involved in fatty acid metabolism in response to nutritional and hormonal signals. The increase of serum insulin has been shown to participate in these responses [6,15-18], and SREBP-1c is the dominant form in adipocytes. Peroxisome proliferator activator receptor-γ2 is a master regulator of adipocyte differentiation and also regulates the expression of lipogenic genes involved in ACC and ACS. The expression of PPAR-γ is stimulated by insulin and SREBP-1 [19,20]. As shown in Fig. 3, the expression of SREBP-1c mRNA was significantly higher in EX-RF rats than in F, RF1, and RF-EX rats. Peroxisome proliferator activator receptor-γ1 mRNA expression was also higher in EX-RF than in C and RF-EX rats, but we did not observe a significant change in PPARγ-2 mRNA expression.

3.4. Plasma insulin and leptin concentration

Because adipocyte mRNA expression is associated with significant changes in plasma hormone and cytokine concentrations [21], we measured plasma insulin and leptin, which are important factors influencing the fatty metabolism (Table 2). Plasma insulin was reduced significantly by fasting

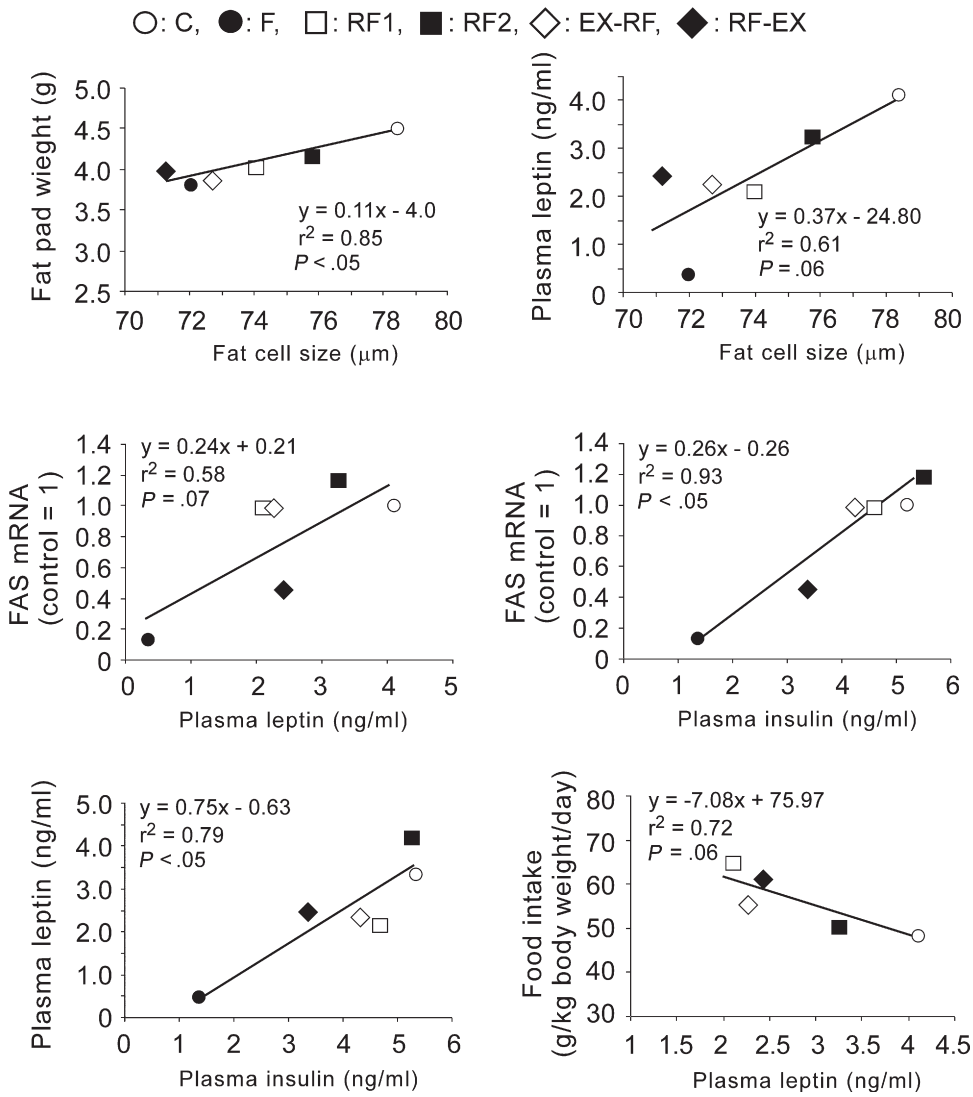

Fig. 4. Correlation between the indicated factors. Values are the mean of the rats under each experimental condition (see legend to Fig. 1).

(F rats) but returned to a level similar to baseline in RF1, RF2, and EX-RF rats. However, plasma insulin was lower in RF-EX rats than in other rats except in F rats. Plasma leptin was also reduced significantly by fasting. The leptin level in RF1, RF2, EX-RF, and RF-EX rats was significantly increased after refeeding compared with that in F rats, but was significantly lower than in C rats. Plasma leptin in RF2 rats returned to a value similar to baseline and was significantly greater than in other rat groups except in C rats.

Both insulin and leptin are thought to regulate the expression of various mRNAs in adipocytes. For example, it has been reported that the expression of SREBP-1c and FAS mRNA is dependent upon the plasma insulin level [6,16]. Moreover, the ability of an adipocyte to secrete leptin may depend on its size. Therefore, we calculated the correlation among the parameters tested. As shown in Fig. 4, a significant relationship ($P < .05$), or a tendency toward a significant relationship ($P < .1$), was found only between the following 6 parameters: fat pad weight vs adipocyte size, leptin vs adipocyte size, FAS mRNA vs leptin, FAS mRNA vs insulin, leptin vs insulin, and food intake vs leptin (this last correlation was calculated without the data from F rats because F rats were not fed). Fat pad weight mainly correlated with adipocyte size ($r^2 = 0.85$). As reported elsewhere, a correlation was found between the plasma leptin level and adipocyte size ($r^2 = 0.61$, $P = .06$). However, when the comparison was restricted to rats under dietary manipulation alone, a strong correlation was found between both parameters ($r^2 = 0.98$). Fatty acid synthase mRNA expression tended to be correlated with plasma leptin ($r^2 = 0.58$, $P = .07$) and was strongly correlated with plasma insulin ($r^2 = 0.93$). A significant correlation was observed between plasma leptin and insulin ($r^2 = 0.79$). Finally, food intake tended to correlate with the leptin level ($r^2 = 0.72$, $P = .06$).

4. Discussion

Dietary manipulation, exemplified by fasting and refeeding, has been shown to affect lipolytic and lipogenic metabolism in adipose tissue [1-7], and exercise after refeeding prevented the refeeding-induced increase in FAS activity in rat liver [8,9]. However, there has been no direct evidence to clarify whether exercise prevents or promotes dietary manipulation–induced changes in either adipocyte morphology or the expression of mRNAs involved in adipocyte metabolic responses. The present study shows that exercise before or after refeeding prevents refeeding-induced restoration of adipocyte size after fasting. In addition, exercise before or after refeeding led to different patterns of expression of metabolic mRNAs: prior exercise enhanced the expression of lipolytic mRNAs (β_3-AR and HSL), whereas exercise after refeeding did not alter the expression of these genes but did induce lower levels of lipogenic mRNAs (FAS, ACC, and ACS) compared with the level found at the respective refeeding day.

However, the reduced expression of β_3-AR mRNA observed after fasting conflicts with prior studies indicating that fasting enhances β_3-AR mRNA [3,4] and the binding amount of [^{3}H]dihydroalprenolol [5]. The reason for this discrepancy is unknown at present. Therefore, although exercise performed before or after refeeding gives rise to distinct effects on lipolytic and lipogenic gene expression, this unresolved discrepancy might limit interpretation concerning changes in β_3-AR mRNA.

The strong correlation between insulin and FAS mRNA expression suggests that the effect of each type of exercise on FAS mRNA expression results from a difference in plasma insulin in the exercising groups. However, we found no significant correlation between insulin and the expression of either ACC or ACS mRNA. Moreover, the different patterns of SREBP-1c and PPAR-γ1 mRNA expression between each exercise group did not coordinate with the observed changes in lipogenic gene expression in each group. Finally, although insulin can down-regulate β_3-AR mRNA expression [4,22], β_3-AR mRNA expression was higher in EX-RF rats whose insulin level was greater than that observed in RF-EX rats. Consequently, factors other than insulin must be considered, such as those possibly involved in the control of the expressions of genes other than FAS under our experimental conditions. Future studies should address the possibility that both negative energy balance and energy availability will differ between fasted rats subjected to exercise before or after refeeding. EX-RF rats exercised under conditions in which liver and muscle glycogen might have been depleted because of the 65-hour fast, and thus, they likely experienced severe negative glucose utilization during exercise and may thus have been forced to depend largely on fat for energy. On the other hand, liver glycogen levels would be expected to be higher in RF-EX than in EX-RF rats because hepatic glucose production, hepatic glycogen levels, and plasma glucose were higher in fasted-refed animals than in normally fed and fasted rats [23,24]. Interestingly, glucose itself has been shown to affect lipogenic mRNA expression [25]. Thus, differences in energy homeostasis and related changes in other hormones, such as glucagon and growth hormone, between each exercise group could result in differences in gene expression between the test groups, although this is only speculation at present.

As discussed above, although there was a strong correlation between insulin and FAS mRNA expression, we found no significant correlation between SREBP-1c mRNA expression and either insulin or FAS mRNA expression. This finding appears to conflict in part with a previous report that fasting- or refeeding-induced changes in FAS mRNA expression may be mediated via insulin and its targeted transcription factor, SREBP-1 [6]. However, a similar discrepancy has also been observed in adipose tissue from obese humans. Caloric restriction did not alter the FAS mRNA level but caused SREBP-1c mRNA to increase [26]. Moreover, Palmer et al [27] have shown that insulin-

stimulated FAS mRNA expression does not always require increased SREBP-1 transcription. It thus appears unlikely that SREBP-1c is the principal factor controlling FAS transcription in adipocytes.

It is well known that there is a definitive correlation between adipocyte size and the plasma level of leptin. In accordance with this, plasma leptin coordinated with adipocyte size and insulin, but a more strong correlation between adipocyte size and plasma leptin was particularly evident among rats subjected to dietary manipulation alone. Thus, plasma leptin levels were quite sensitive to changes in adipocyte size in rats that experienced dietary manipulation alone. However, a combination of exercise with dietary manipulation blunted this correlation. Although there is a consensus that exercise does not immediately modulate leptin levels [28], a combination of exercise with dietary manipulation, either of which is clearly a different physiologic condition, might induce a change in plasma leptin, at least independently of adipocyte size.

A weak (P = .06) but good correlation between plasma leptin and food intake suggests a role for leptin in controlling food intake. However, our data do not exclude a role for hormonal factors other than leptin in regulating food intake. Indeed, a recent study has shown that exercise increased plasma ghrelin in fed rats [29]. Because ghrelin can increase food intake, a possible increase in the ghrelin level could also conceivably have been involved in the greater food intake of RF-EX rats than RF2 rats. On the other hand, food restriction appears to reduce the expression of corticotropin-releasing hormone (CRH), which inhibits food intake [30,31], in the paraventicular nucleus [31], but food-restricted exercising rats exhibited increased CRH expression in the hypothalamic arcuate nucleus proopiomelanocortin and paraventicular nucleus compared with restricted sedentary rats [32]. Thus, in EX-RF rats that are subjected to exercise during fasting, it is possible that the inhibitory effect of CRH on food intake is maintained during subsequent refeeding.

In conclusion, exercise, either before or after refeeding, limited the refeeding-induced restoration of adipocyte size after fasting. However, the effect of the exercise on metabolic mRNA expression in adipocytes was dependent upon the timing relative to the refeeding. Exercise before refeeding enhanced the expressions of β_3-AR and HSL mRNA, whereas exercise after refeeding inhibited expression of FAS mRNA. Thus, exercise before refeeding might lead to enhanced adipocyte lipolysis, whereas exercise after refeeding might inhibit adipocyte lipogenesis. Both of these effects prevent the refeeding-induced restoration of adipocyte size after fasting. However, exercise before refeeding, but not after it, blunted food intake after exercise. Further study to explore more definite mechanism and conclusion might require experiments to identify protein levels and activities of the genes tested. In addition, our experimental model represents abnormal dietary manipulation, which may limit its extrapolation to human beings. However, to prevent the refeeding-induced restoration of adipocyte size, exercise after refeeding would be preferable to that before refeeding because it may give rise to less stress on food intake during refeeding postfasting compared with exercise performed before refeeding postfasting.

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