ELSEVIER

Relationships among serum triacylglycerol, fat pad weight, and lipolysis in iron-deficient rats

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We studied the relationships among serum triacylglycerol (TG), fat pad weight, and lipolytic response to norepinephrine (NE) in iron-deficient rats. We used male Sprague-Dawley International Golden Standard rats. The rats were randomly divided into four groups: two iron-adequate groups for 1 week (1A) and 5 weeks (5A), and two iron-deficient groups for 1 week (1D) and 5 weeks (5D), based on the AIN-93G diet. Iron-deficient treatment caused a significant decrease in hemoglobin (Hb) and hematocrit (Hct) values and an increase in relative heart weight in 1D and 5D rats. Although serum TG was not affected by the 1-week iron-deficient treatment, it was significantly increased by 5-week iron-deficient treatment. The 1-week iron-deficient treatment significantly decreased the relative weight of the retroperitoneal fat pads, but not that of the epididymal fat pads. On the other hand, the 5-week iron-deficient treatment significantly decreased the relative weight of both fat pads; the degree of decrease was 41% and 32% for retroperitoneal and epididymal fat pads, respectively. Basal lipolysis significantly decreased in the epididymal adipocytes from 1D rats, whereas lipolytic response to NE markedly increased. No effect due to the 5-week treatment on basal lipolysis was observed in either retroperitoneal or epididymal adipocytes. In addition, lipolytic response to NE significantly increased in the retroperitoneal, but not the epididymal adipocytes. These results demonstrate that the effects of an iron-deficient diet on fat pad weight are different, depending on the duration of the treatment and the location of fat pads. In addition, iron deficiency-caused hypertriacylglycerolmia may be predominantly related to the increase in lipolysis in retroperitoneal rather than in epididymal adipocytes. The data further show that the increase in lipolysis of epididymal adipocytes occurs in the earlier stage prior to a severe iron-deficient state. (J. Nutr. Biochem. 11:455–460, 2000)

Keywords: rats; iron deficiency; norepinephrine; adipocytes; lipolysis

Introduction

Iron deficiency is a nutritional problem of worldwide importance, contributing greatly to human and animal morbidity. The most apparent physiological consequences of iron deficiency are attributable to anemia. Although individuals with iron deficiency anemia are not at their limits of O_2 consumption at rest, it might be to reasonable to shift more efficient substrates concerning the O_2 consumption.[1] Under these conditions, glucose might be a preferred metabolic fuel in iron deficiency anemia.[1–4] Davies et al.[2] and Linderman et al.[3] showed that levels of blood glucose and lactate were higher in iron-deficient rats than controls during both rest and submaximal exercise. For this reason, glucose, which can be utilized to produce ATP without benefit of mitochondrial electron transport and

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which provides a greater energy yield per O_2 consumed than fat, might be a preferred substrate in iron-deficient status.

On the other hand, the association between iron deficiency and an increase in blood triacylglycerol (TG) has been described by many investigators.[5–10] As for endogenous TG synthesis, experimental results on lipogenesis from labeled glucose by liver in iron-deficient rats are contradictory: Amine et al.[5] observed decreasing levels and Sherman et al.[6] reported increasing levels. As for TG uptake, an electron microscopic study showed an increase in the number of lipid droplets in the skeletal muscle of iron-deficient rats.[11] For this reason, the reductions of tissue carnitine levels and its functions in an iron-deficient state have been reported by several investigations.[12–14] We also showed that iron deficiency provokes increased serum TG in strains of Fischer-344, Sprague-Dawley, and Wistar male rats (0.74 vs. 0.85, 1.17 vs. 3.83, and 0.97 vs. 3.84 mmol/L for iron-adequate vs. iron-deficient, respectively).[15]

Iron-deficient anemic rats result in an elevation of norepinephrine (NE) levels in blood and urine.[16,17] NE stimulates cardiac output. As a consequence, arterial O_2 transport is sufficient in resting iron-deficient rats.[4] Because NE has a stimulant effect on lipolysis, it is probable that the increase in blood NE level due to iron deficiency leads to the increase in lipolysis. Notably, we have observed the decreased relative retroperitoneal fat pad weight in strains of Fischer-344, Sprague-Dawley, and Wistar male rats (1.28 vs. 0.53, 1.75 vs. 0.59, and 1.62 vs. 0.96 g/100 g body weight for iron-adequate vs. iron-deficient, respectively).[15] To our knowledge, there is no information on lipolysis during β-adrenergic stimulation in iron-deficient animals. We hypothesized that the increase in serum TG accumulation occurs as the result of enhanced lipolysis by NE. Our intention in this investigation was to observe the relationships among serum TG, fat pad weight, and lipolysis in iron-deficient rats.

Methods and materials

Animals

We used Male Sprague-Dawley International Golden Standard rats (SD-IGS) from Japan Charles River Inc. (Kanagawa, Japan). The rats were randomly divided into four groups: two iron-adequate groups (50 mg/kg diet) for 1 week (1A; $n = 13$) and for 5 weeks (5A; $n = 12$), and two iron-deficient groups (8 mg/kg diet) for 1 week (1D; $n = 13$) and for 5 weeks (5D; $n = 12$), respectively, based on the AIN-93G diet,[18] as shown in *Table 1*. Rats were housed individually in hanging wire-mesh cages in a room with the temperature controlled at 23 ± 1°C and a 12:12-hr light/dark cycle. They were given free access to food and distilled water. Daily food intake and body weights were recorded. The animals were fasted overnight before the experiments. Blood for hemoglobin (Hb) and hematocrit (Hct) measurements was obtained from the tail vein before the decapitation. All rats were decapitated under light etherization and blood was obtained for biochemical measurements according to the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" of the Japan Society of Nutrition and Food Sciences (Notification of the Japanese Prime Minister's Office).

Table 1 Composition of basal diet*

Ingredient (g/kg)	Iron-adequate	Iron-deficient
Casein†	200.0	200.0
L-cystine‡	3.0	3.0
α-cornstarch†	529.5	529.5
Sucrose†	100.0	100.0
Cellulose†	50.0	50.0
Soybean oil†	70.0	70.0
AIN-93G mineral mixture†	35.0	35.0§
AIN-93G vitamin mixture†	10.0	10.0
Sodium choline‡	2.5	2.5

*Based on the AIN-93G (Reeves et al.[18]).
†Purchased from Oriental Yeast Co. (Tokyo, Japan).
‡Purchased from Wako Pure Chemical Co. (Osaka, Japan).
§Ferric citrate was excepted.

Biochemical measurements

Hb, TG, and glucose concentrations were determined with commercial kits (Hemoglobin-Test Wako, TG E-Test Wako, and Glucose CII-Test Wako, respectively, Wako Pure Chemical Co., Osaka, Japan). Hct was determined by centrifugation in a capillary tube system.

Preparation and incubation of intact adipocytes

Epididymal and retroperitoneal fat pads were quickly excised. Adipocytes were isolated using a modification of the method of Rodbell.[19] Briefly, fat pads (2–3 g) were incubated at 37°C for 30 min in plastic vials in a medium containing collagenase type 1 (5 mg/mL, Worthingthon Biochemical) plus (in mmol/L) 100 NaCl, 4.73 KCl, 1.28 $CaCl_2$, 1.18 $MgSO_4$, 1.18 KH_2PO_4, 5 glucose, 10 HEPES, and 3% (wt/vol) fatty acid-free bovine serum albumin (Sigma Chemicals). The medium was maintained at pH 7.4 under an atmosphere of 95% O_2 and 5% CO_2. As recommended by Honnor et al.,[20] the medium contained 200 nmol/L adenosine (Merck) to limit glycerol production during collagenase incubation. After incubation, the contents of the vials were immediately filtered by nylon mesh with pore size 250 μm and centrifuged for 30 sec at 170 × *g*. The supernatant cell layer was then washed three times with the medium described above. Pooled adipocytes were assigned to the indicated experiments as occasion demanded. Adipocytes (10–25 × 10^4 cells/mL) were incubated with or without the NE (Sankyo Co.) in plastic tubes at 37°C for 30 min. After incubation, the reaction was stopped by immersing into an ice-water bath, the top layer of adipocytes was aspirated, and the cell-free incubation medium was assayed for glycerol as an index of lipolysis. The average number of cells was determined using the method of Izawa et al.[21] Briefly, a 50-μL aliquot of the initial cell suspension was diluted with 450 μL of medium. Three of these suspensions were used for cell counting under a microscope. A 5-μL portion of one suspension was placed on a hemacytometer slide, and triplicated counts were performed for each slide. This procedure was repeated three times for each suspension. The average number of adipocytes per 5 μL represents the number of adipocytes per 0.5 μL of the initial suspension. Glycerol was measured with a commercial kit (F-kit Glycerol; Boehringer Mannheim).

Data analyses

All data are expressed as mean ± SEM. The significance of difference between means within the same feeding term was

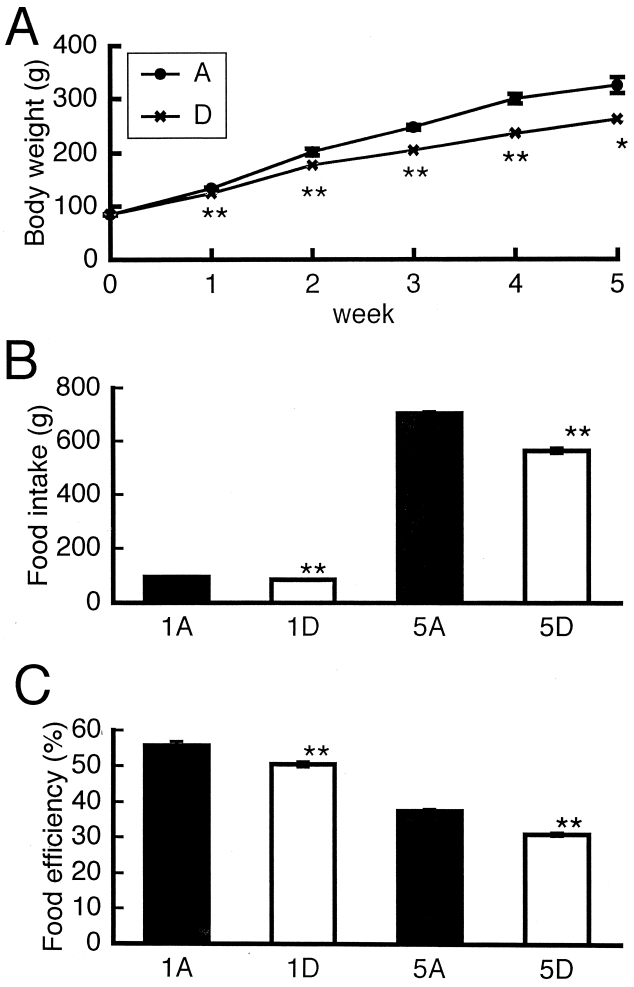

Figure 1 Growth rates (A), food intake (B), and food efficiency (C) in iron-adequate and iron-deficient rats. Data are means ± SEM (1A, 1D, 5A, and 5D were 13, 13, 12, and 12 rats, respectively). Data at 0 and 1 week in growth rates (A) are sum of same dietary treatment ($n = 25$). Significant differences within the same treatment term: $^{*}P < 0.05$; $^{**}P < 0.01$.

determined by Student's *t*-test. When variances were not homogeneous in the F-test, data were analyzed by Mann-Whitney's U-test. *P*-values < 0.05 were considered significant. In figures where SEM bars are not shown, they are within the symbols.

Results

Growth curve, food intake, and food efficiency

Figure 1 shows growth curves (A), food intake (B), and food efficiency (C) in each group. The points of the growth curves at 0- and 1-week data included 1-week fed rats ($n = 25$). Depressive effects of an iron-deficient diet on these data (growth curves, food intake, and food efficiency) in 1D rats were slight but significant. Therefore, all data in both iron-deficient rats were significantly lower than iron-sufficient rats.

Biochemical markers and relative tissue weights

Table 2 shows the changes in biochemical markers (Hb, Hct, TG, and glucose values) and relative tissue (heart, epididymal, and retroperitoneal fat pads) weights in each groups. In the 1-week treatment group, Hb and Hct values were already significantly decreased in 1D rats. After 5-week treatment, these values were significantly lower in 5D than in 5A rats. At the same time, the effects of an iron-deficient diet were demonstrated not only directly by iron profiles (Hb and Hct), but also by other indirect variables; for example, relative heart weight and serum glucose values in 1D and 5D rats were significantly greater than those in 1A and 5A rats within the same feeding term. Moreover, the relative weight of retroperitoneal fat pads in 1D rats significantly decreased, but that of epididymal fat pads did not. On the other hand, the relative weight of both fat pads significantly decreased in 5D rats. Serum TG concentrations in 1A and 1D rats were similar, but those in 5D rats were significantly higher than in 5A rats.

Lipolytic responses in 1-week-treated rats

Figure 2 shows the dose-response curves for NE-stimulated lipolysis in epididymal adipocytes from 1-week-treated rats. Although the basal lipolysis significantly decreased in 1D rats (*Figure 2A*), the lipolytic response (presented in % basal) to NE significantly increased in 1D rats at 1 and 10 μmol/L of NE (*Figure 2B*). These experiments were not

Table 2 Biochemical markers and relative tissue weights

	1-week-fed rats ($n = 13$)			5-week-fed rats ($n = 12$)		
	Iron-adequate	Iron-deficient	*P*	Iron-adequate	Iron-deficient	*P*
Biochemical markers						
Hemoglobin (g/L)	121 ± 2	74 ± 3	<0.01	152 ± 3	49 ± 2	<0.01
Hematocrit (%)	42.9 ± 0.6	27.0 ± 0.9	<0.01	48.2 ± 0.6	21.5 ± 0.8	<0.01
Triacylglycerol (mmol/L)	0.69 ± 0.03	0.64 ± 0.05	>0.05	0.91 ± 0.11	2.08 ± 0.48	<0.01
Glucose (mmol/L)	4.6 ± 0.2	5.8 ± 0.3	<0.01	5.7 ± 0.3	7.0 ± 0.3	<0.01
Relative tissue weights (g/100 g body weight)						
Heart	0.449 ± 0.013	0.499 ± 0.016	<0.05	0.334 ± 0.005	0.558 ± 0.025	<0.01
Epididymal fat pads	0.333 ± 0.021	0.296 ± 0.019	>0.05	1.241 ± 0.103	0.849 ± 0.049	<0.01
Retroperitoneal fat pads	0.257 ± 0.026	0.168 ± 0.020	<0.05	1.156 ± 0.103	0.678 ± 0.057	<0.01

Data are expressed as mean ± SEM.

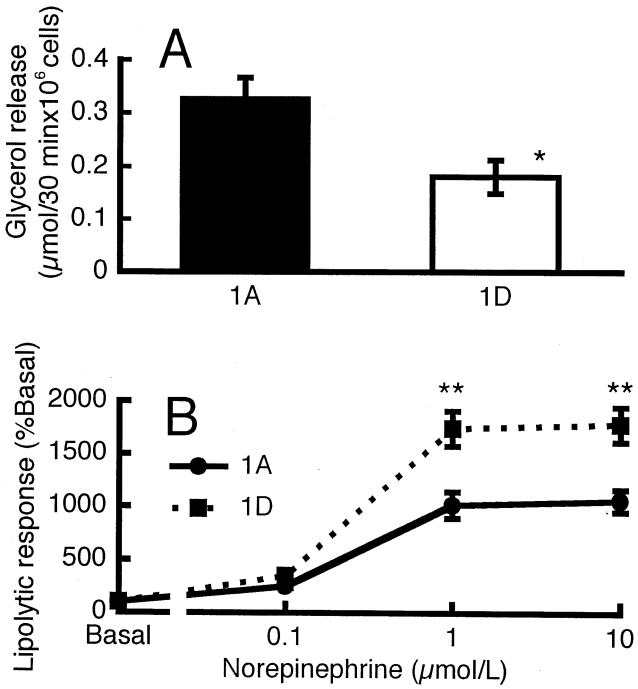

Figure 2 Lipolytic responses of epididymal adipocytes from 1-week-treated iron-adequate diet (1A) and iron-deficient diet (1D) rats to norepinephrine. Adipocytes (10–25 × 10^4 cells) were incubated with absence (A) or presence (B) of norepinephrine at 37°C for 30 min. Reactions were terminated, and glycerol release was determined as described in the Methods and materials section. Data are means ± SEM of triplicate determinations from four separate experiments. Significant differences of 1A vs. 1D within the same concentration: $^*P < 0.05$; $^{**}P < 0.01$.

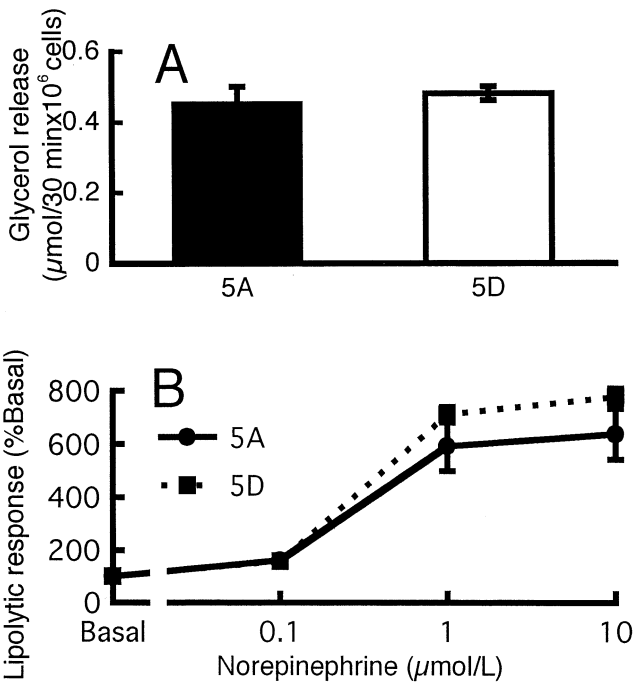

Figure 3 Lipolytic responses of epididymal adipocytes from 5-weeks-treated iron-adequate diet (5A) and iron-deficient diet (5D) rats to norepinephrine. Adipocytes (10–25 × 10^4 cells) were incubated with absence (A) or presence (B) of norepinephrine at 37°C for 30 min. Reactions were terminated, and glycerol release was determined as described in the Methods and materials section. Data are means ± SEM of triplicate determinations from four separate experiments.

carried out on retroperitoneal fat pads because we could not obtain sufficient cells from 1-week-fed rats due to destruction of the cells during isolation procedure.

Lipolytic responses in 5-week-treated rats

In 5-week-treated rats, *Figure 3* and *Figure 4* show the dose-response curves for NE-stimulated lipolysis in epididymal and retroperitoneal adipocytes, respectively. Basal lipolysis in both epididymal and retroperitoneal adipocytes from 5D and 5A rats showed no significantly difference (*Figures 3A and 4A*). Lipolytic response to NE in the epididymal adipocytes was also unaffected by 5-week treatment (*Figure 3B*). As shown in *Figure 4B*, NE-stimulated lipolysis in retroperitoneal adipocytes from 5D rats was significantly higher ($P < 0.01$) than that in 5A rats at all concentrations examined.

Discussion

Many previous reports indicated that iron deficiency provokes alterations in energy metabolism in animal experiments. In our data, the levels of serum glucose in both 1D and 5D rats was significantly higher than those in both 1A and 5A rats. This is consistent with previous reports.[1,22–24] The preferential use of glucose as a fuel in iron-deficient rats appears to minimize the consequences to limitations in aerobic metabolism.[1] This elevated blood glucose may stimulate the hypothalamic "satiety center" by increasing glucose utilization in this region, which inhibits the hypothalamic "feeding center." Therefore, food intake of 1D rats was depressed (slightly but significantly) compared with 1A rats. In addition, a greater whole-body catabolism of glucose in iron-deficient rats would present a less efficient source of fuel than fat for the increase and maintenance of body weight.[1] Stangl and Kirchgessner[25] observed that the gain in body weight was lower in iron-deficient rats (receiving 9 mg iron/kg diet for 5 weeks) than the corresponding pair-fed controls. Thus, alterations in glucose metabolism may partly explain the decrease in growth rate and lower body weights of iron-deficient rats.[1]

It is interesting that iron deficiency provokes alterations in both serum TG concentrations and relative fat pad weight in the present investigation. This is consistent with our previous reports.[15] Additionally, the results of the present study suggest that the effects of an iron-deficient diet on fat pad weight depend on the duration of treatment, and that the decrease in relative weight is greater in retroperitoneal than in epididymal fat pads. This may be explained by evidence that in vitro lipolytic response to NE in retroperitoneal adipocytes from 5D rats is higher than from 5A rats (see below).

Dillmann et al.[16] reported that iron deficiency provokes an increase in blood NE. In addition, Beard et al.[17] indicated that heart NE content significantly increased 3 days after the similar treatment of the present investigation. In our results, Hb and Hct values in 1D rats were already decreased (60%

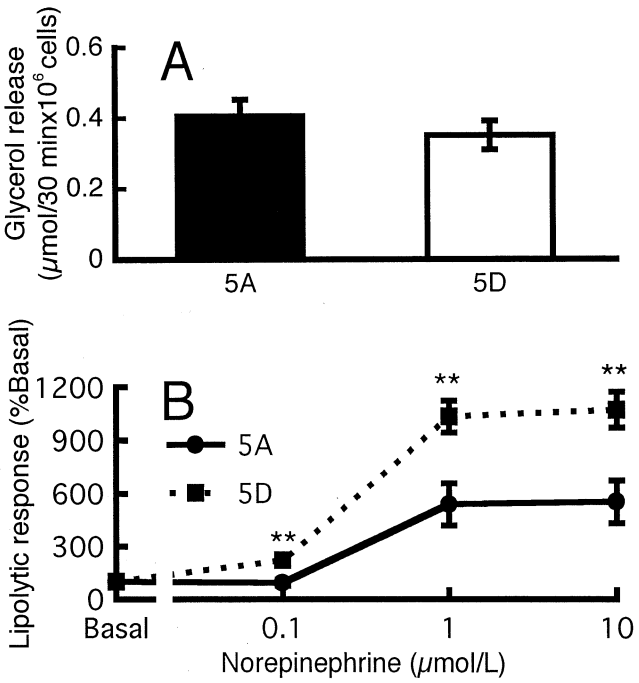

Figure 4 Lipolytic responses of retroperitoneal adipocytes from 5-weeks-treated iron-adequate diet (5A) and iron-deficient diet (5D) rats to norepinephrine. Adipocytes ($10–25 \times 10^4$ cells) were incubated with absence (A) or presence (B) of norepinephrine at 37°C for 30 min. Reactions were terminated, and glycerol release was determined as described in the Methods and materials section. Data are means ± SEM of triplicate determinations from four separate experiments. Significant differences of 5A vs. 5D within the same concentration: $^{**}P < 0.01$.

and 63% of those obtained from 1A rats, respectively). Therefore, relative heart weight in 1D rats also increased to 126% of that of 1A rats. In the 5-week treatment, Hb and Hct values in 5D rats were very low and were 32% and 45% of those obtained from 5A rats, respectively. Relative heart weight in 5D rats also increased to 167% of that of 5A rats. We did not measured blood NE level directly in this study, but it is probable that NE was elevated in both 1D and 5D rats deducing from the Hb, Hct, and relative heart weight.

Because NE has a stimulant effect on lipolysis, it is probable that the increase in blood NE level due to iron deficiency leads to the increase in lipolysis. Our presented data lack serum nonesterified fatty acids (NEFA) as an index of in vivo lipolysis because we had insufficient sample (serum) for the measurement of NEFA. We focused on changes in serum TG in order to study the effect of iron deficiency on the movement of blood lipids. The explanation for the relationship between elevated serum TG concentration and iron deficiency is not so clear. To date, it has not been demonstrated conclusively whether iron-deficient treatment has an effect on NE-induced lipolysis. If adipose tissues in iron-deficient rats are continuously exposed to high concentrations of NE, the adipocyte response to NE (through β-adrenergic receptors) is thought to vary with exposure time.

Regarding the effect of iron deficiency in the earlier stage, Sherman[10] reported that despite the decrease in Hb and Hct values, the increase in serum TG did not occur in weanling rats (21 days of age) fed an iron-deficient diet for 9 days. Our data also show that serum TG concentration was not significantly changed in 1D rats. This is consistent with the results of Sherman.[10] The relative weight of epididymal fat pads in 1D rats was not affected, whereas that of retroperitoneal fat pads significantly decreased. Although the 1-week treatment significantly decreased basal lipolysis in epididymal adipocytes, the reasons for this decrease are not apparent. However, the lipolytic response to NE in epididymal adipocytes from 1D rats was greater than that in 1A rats at ≥1μmol/L of NE. The reason that the decrease in relative weight of retroperitoneal fat pads was not reflected in the serum TG levels may be related to the positive utilization of lipids together with glucose oxidation.

In contrast, the serum TG values in 5D rats were markedly higher than those in 5A rats (5D rats, 2.07 mmol/L vs. 5A rats, 0.91 mmol/L). The relative weight of fat pads in 5D rats decreased not only for retroperitoneal but also for epididymal fat pads; the decrease in relative weight was larger in retroperitoneal than in epididymal fat pads (32% and 41% for epididymal and retroperitoneal fat pads, respectively). In addition, NE-stimulated lipolytic responses in epididymal adipocytes from 5-week-treated rats were similar, whereas lipolytic responsiveness in retroperitoneal adipocytes was greater in 5D than in 5A rats. These results suggest that the lipolytic response to NE is different between epididymal and retroperitoneal adipocytes. Therefore, it seems that increased serum TG concentration in 5D rats may be more efficient to the increased lipolysis of retroperitoneal than epididymal adipocytes.

We did not assess the characteristics of β-adrenergic receptors in the present study. Although speculative, the increased response that was observed in epididymal adipocytes in 1D rats and in retroperitoneal adipocytes in 5D rats may be related to the up-regulation of β-adrenergic receptors. On the other hand, the fact that lipolytic responsiveness did not increase in epididymal adipocytes in 5D rats may be related to receptor desensitization.

It is widely accepted that the lipolytic response of adipocytes is mediated through a cyclic AMP (cAMP)-dependent process. As described above, iron-deficient treatment had effects on lipolysis via β-adrenergic receptors. Thus, it is of interest to examine whether iron-deficient treatment has an effect on the post-cAMP event. The effects of iron-deficient treatment on lipolytic response to dibutyryl cAMP (5 mmol/L) were similar to those of NE stimulation (data not shown). These results suggest that iron-deficient treatment has an effect on the post-cAMP event. Taken together, it seems that the effects of iron deficiency on lipolysis vary among fat pads and that the effects occur in all lipolytic mechanisms, but only through activation of a specific step.

Our findings are the first indication that iron-deficient treatment induces changes in the lipolysis of epididymal adipocytes in the earlier stage. Such an early change in cellular responses was reported in Ca^{2+} regulation observed in pancreatic acinar cells from acute streptozotocin-diabetic rats.[26] Similarly, changes in lipid metabolism and insulin effects in adipocytes are observed in rats fed a (n-3) or (n-6) polyunsaturated fatty acids-enriched diet for 1 week.[27] These changes that occur in the earlier stages following

treatment may be an initial step involved in the adaptation process.

The whole-body fuel substrate metabolism in severe iron-deficient rats brings about elevated glucose and TG concentrations. Borel et al.[22] suggested that in the most extreme scenario of prolonged hyperglycemia in iron deficiency anemia, physiologic and metabolic processes were similar to those that were observed in diabetes mellitus. However, Farrell et al.[23] and Borel et al.[24] demonstrated that although iron-deficient rats presented elevated blood insulin compared with iron-adequate rats, peripheral insulin responsiveness in severely and moderately iron-deficient anemic rats was higher than iron-sufficient rats. Even so, Storz et al.[28] demonstrated an important role for NEFA as the major insulin-resistance-inducing agent in skeletal muscle cells. It is helpful that iron deficiency would result in insulin resistance: up-regulated lipolytic responses, hyperinsulinemia,[23,24] and accumulated lipid droplet in skeletal muscle.[11] Further, insulin has antagonistic NE action to adipocyte lipolysis and agonistic action to TG synthesis from NEFA by hepatocytes. These insulin stimulations are opposed to NE stimulations, especially in adipocytes.

In this study, we could not present direct evidence that the increase in TG synthesis and/or the decrease in TG uptake are provoked in target tissues. Therefore, further investigation regarding the effects of iron deficiency on fuel metabolism would provide valuable information to explain the mechanism of hypertriacylglycerolmia.

In conclusion, the effects of an iron-deficient diet on fat pad weight are different, depending on the duration of the treatment and location of the fat pads. In addition, hypertriacylglycerolmia provoked by iron deficiency may be predominantly related to the increase of lipolysis in retroperitoneal rather than that in epididymal adipocytes. The data show further that the increase in lipolysis in epididymal adipocytes occurs in an earlier stage prior to the severe iron-deficient state.

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