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Characteristics of lipolysis in white adipose tissues of SHR/NDmc-cp rats, a model of metabolic syndrome

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Abstract

This study shows the characteristics of hormone-dependent lipolysis in white adipose tissues from corpulent spontaneously hypertensive rats (SHR/NDmc-cp(cp/cp)). The glycerol-releasing activity on addition of norepinephrine (NE) and corticotropin (ACTH) was diminished in slices of epididymal, retroperitoneal, and mesenteric adipose tissues from cp/cp rats compared with those from Wistar Kyoto rats and lean spontaneous hypertensive rats (SHR/NDmc-cp(+/+)). 8-Bromo-cyclic adenosine monophosphate had a slight effect on lipolysis in epididymal, retroperitoneal, and mesenteric adipose tissues from cp/cp rats, and addition of NE and ACTH resulted in a slight accumulation of cyclic adenosine monophosphate in epididymal adipose tissue from cp/cp rats. Therefore, the alteration of hormone-dependent lipolysis-related genes was analyzed using quantitative real-time polymerase chain reaction. It was found that the expression of β_3-adrenergic receptor, melanocortin 2 receptor, hormone-sensitive lipase, and perilipin messenger RNAs was limited in epididymal, retroperitoneal, mesenteric, and subcutaneous adipose tissues from cp/cp rats compared with +/+ rats. These results indicate that in white adipose tissue from cp/cp rats, the diminished lipolytic response to NE and ACTH may be caused by impaired expression of β_3-adrenergic receptor, melanocortin 2 receptor, hormone-sensitive lipase, and perilipin.

1. Introduction

Obesity is one of the metabolic abnormalities of metabolic syndrome along with hypertension, hyperlipidemia, hyperglycemia, and hyperinsulinemia. Obesity is characterized by increases in body and adipose tissue weight resulting from increasing numbers of adipocytes with diminished lipolytic responses to hormones [1].

Adipocytes are characterized as synthesizing and releasing several physiologically active materials called adipocytokines. Leptin, a major adipocytokine, interacts with hypothalamic receptors to regulate appetite and energy expenditure. Genetic defects in the leptin–hypothalamic feedback loop result in obesity in several rodent models.

Corpulent spontaneously hypertensive rats (SHR), such as Koletsky rats, SHR/N-cp rats, and SHR/NDmc-cp rats, in which the leptin receptor gene is knocked out, have been shown to develop obesity as well as hypertension, hyperlipidemia, and hyperinsulinemia. These rats exhibit metabolic and histopathologic characteristics associated with metabolic disorders in humans, such as increases of body and adipose tissue weights [2] accompanying hypertension and hypercardia [3], diabetes [4-6], and hyperlipidemia [7].

Little is known about the lipolytic response to hormones in adipose tissues from corpulent SHR. Only a decreased lipolytic response to catecholamines was demonstrated in adipose tissues from genetically obese rats, Zucker rats, and obese rats fed with a diet [8,9]. However, in adipose tissues from SHR, the lipolytic response to corticotropin (ACTH), but not catecholamines, was reported to be greater than that in normotensive rats [10]. Therefore, it is not possible to estimate the changes in lipolytic response in adipose tissues of corpulent SHR. Because adipocytes of corpulent SHR are enlarged in size [2], the lipolytic response to hormone stimulation appears to be diminished.

To better understand the diminished lipolytic response, we studied in vitro the lipolytic response to ACTH and norepinephrine (NE), and the lipolysis-related gene expression of adipose tissues from SHR/NDmc-cp rats compared with spontaneously hypertensive and Wistar Kyoto (WKY) rats.

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2. Materials and methods

2.1. Animals

Male corpulent SHR (SHR/NDmc-cp(cp/cp)), lean spontaneous hypertensive rats (SHR/NDmc-cp(+/+)), and WKY rats were purchased from SLC (Shizuoka, Japan). The animals were housed in cages at 23°C ± 1°C and 55% ± 5% relative humidity with lighting from 8:00 AM to 8:00 PM daily. Throughout the experimental period, all animals were fed with a normal diet (CE-2; CLEA, Tokyo, Japan). The body weight of cp/cp rats increased with age and was gradually heavier than that of WKY rats after 6 weeks of age. On the other hand, the body weight difference between +/+ and WKY rats was almost same with age. Other physiologic parameters including blood pressure, and serum insulin and lipid levels were already reported previously [11]. All animals at 12 weeks of age were weighed, and blood pressure was measured by the tail cuff method using MK-2000 (Muromachi Kikai, Tokyo, Japan). All the animals used in this study were treated in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Mukogawa Women's University (Nishinomiya, Japan).

2.2. Cell size, and triglyceride and DNA contents

Adipose tissue was fixed in paraformaldehyde, embedded in paraffin, cut into 20-μm sections, and stained with hematoxylin and eosin (Fig. 1). The adipocyte diameter was measured by microscopic examination with counting of 60 adipocytes per each of 3 slides and then averaged.

Fifty milligrams of adipose tissue was homogenized in 600 μL of chloroform-methanol (2:1). The solvent was removed and evaporated. The residue was resolved in 1% Triton-X. The sample was used to determine the amount of triglyceride by the colorimetric method [12].

One hundred milligrams of adipose tissue was homogenized in 600 μL of 10 mmol/L Tris buffer, pH 8.0, containing 0.5 mol/L NaCl and 0.1% sodium dodecyl sulfate. The sample was treated with 10 μg/mL of RNase. DNA was extracted using the standard phenol-chloroform extraction method. DNA pellets were resolved in 10 mmol/L NaOH. DNA content was calculated from the absorbance of this solution at 260 nm (OD_{260}) taking the OD_{260} of a 50 μg/mL DNA solution as 1.

2.3. Lipolysis

After decapitation, epididymal, retroperitoneal, mesenteric, and subcutaneous adipose tissues from WKY, +/+, and cp/cp rats at 12 weeks of age were removed and rinsed in a 0.9% NaCl solution. Connective tissues and blood vessels were removed from the adipose tissues. One hundred milligrams of the adipose tissue was cut into small pieces and suspended in 900 μL of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1.15 mmol/L calcium, 3% bovine serum albumin, 10 mmol/L glucose, and 2 mmol/L adenosine triphosphate. After the addition of 100 μL of NE (Nacalai Tesque, Kyoto, Japan) or ACTH (Sigma, St Louis, MO) solution, the samples were incubated with shaking at 37°C under an atmosphere of 5% CO_2–95% O_2 for 60 minutes. The incubation was stopped by cooling on ice. The amount of glycerol released into the incubation medium was determined using the colorimetric method [12].

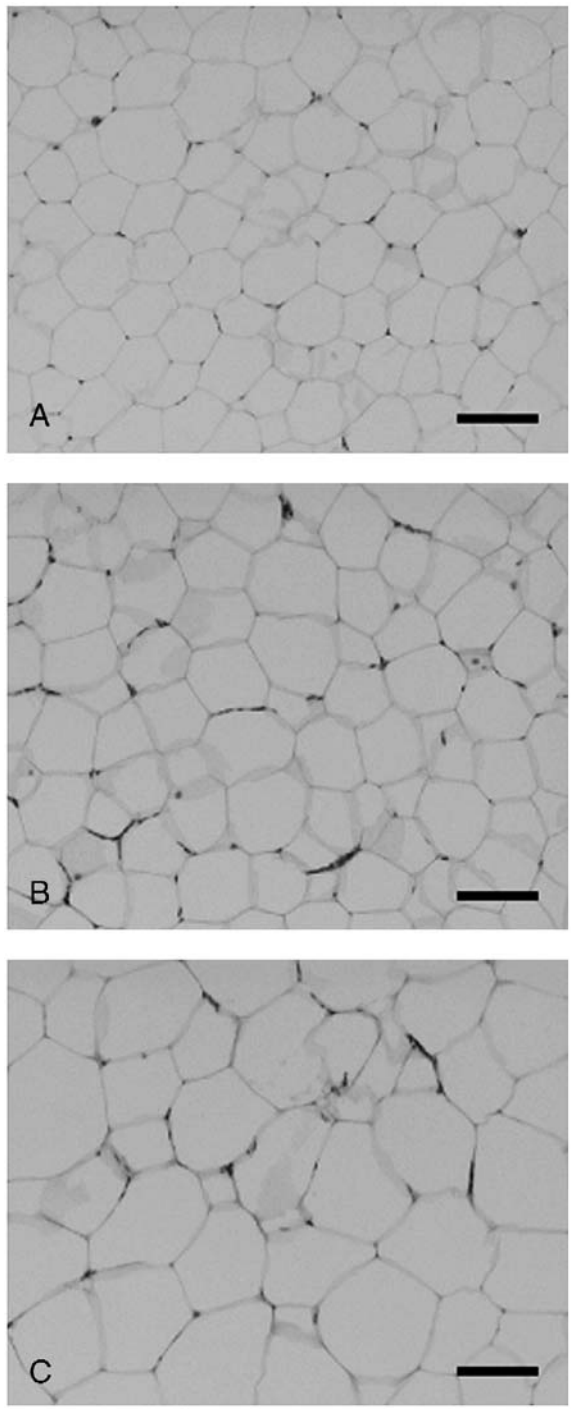

Fig. 1. Hematoxylin and eosin staining in epididymal adipose tissues from WKY (A), SHR/NDmc-cp(+/+) (B), and SHR/NDmc-cp(cp/cp) rats (C) (scale bar = 100 μm).

Table 1
Physiologic parameters and characteristics of white adipose tissue in WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats

	WKY	+/+	cp/cp
Body weight (g)	344.3 ± 11.0	335.3 ± 5.4	451.0 ± 28.0*,†
Blood pressure (mm Hg)	106.8 ± 14.4	160.0 ± 11.3*	143.7 ± 18.6*
Adipose tissue			
Weight (g)			
Epididymal	4.94 ± 0.32	3.40 ± 0.26*	8.76 ± 0.70*,†
Retroperitoneal	6.05 ± 0.53	4.50 ± 0.23	13.55 ± 1.92*,†
Mesenteric	2.83 ± 0.26	2.65 ± 0.18	6.61 ± 0.86*,†
Subcutaneous	8.81 ± 0.82	8.52 ± 0.53	42.12 ± 1.78*,†
Cell size (μm)			
Epididymal	80.6 ± 5.5	86.7 ± 5.1	140.9 ± 3.7*,†
Retroperitoneal	89.8 ± 1.5	88.9 ± 8.8	136.1 ± 8.4*,†
Mesenteric	67.5 ± 4.7	72.0 ± 2.8	120.4 ± 9.3*,†
Subcutaneous	72.3 ± 6.5	75.1 ± 6.2	137.2 ± 15.6*,†
DNA content (μg/mg tissue)			
Epididymal	0.138 ± 0.007	0.123 ± 0.002*	0.100 ± 0.007*,†
Retroperitoneal	0.126 ± 0.012	0.110 ± 0.009	0.101 ± 0.012*
Mesenteric	0.216 ± 0.014	0.194 ± 0.010*	0.135 ± 0.016*,†
Subcutaneous	0.428 ± 0.107	0.290 ± 0.157	0.121 ± 0.020*
Triglyceride content (μmol/mg tissue)			
Epididymal	1.06 ± 0.06	1.04 ± 0.08	1.12 ± 0.08
Retroperitoneal	0.99 ± 0.07	1.01 ± 0.08	0.99 ± 0.11
Mesenteric	0.89 ± 0.04	0.90 ± 0.08	1.07 ± 0.09*,†
Subcutaneous	0.72 ± 0.25	0.61 ± 0.10	1.06 ± 0.10*,†

Values are expressed as mean ± SD (n = 4).
* $P < .05$ vs WKY.
† $P < .05$ vs +/+.

In addition, the effects of 5 mmol/L 8-bromo-cyclic adenosine monophosphate (cAMP) were also investigated.

Although we tried to prepare fat cell suspension from adipose tissues of cp/cp rats according to Rodbell [13], we could not obtain a representative fat cell because of cell loss during the isolation process [14,15], which was previously reported in isolated cells from adipose tissue of JCR:LA-cp (cp/cp) rats by McArthur et al [16]. However, Rodbell [13] indicated that the effects of hormone-stimulated lipolysis in intact adipose tissue were the same as those observed in the free fat cells. Because DNA content was reported to reflect more the number of adipocytes than lipid content and wet tissue weight [17,18], we have calculated the rate of glycerol release per DNA as an index of rate of lipolysis. However, the index based on DNA content possibly includes changes caused by the alteration of the stromal-vascular cell number of adipose tissues [13,19]. Accordingly, the comparisons between various strains were performed with the ratio of the hormone-stimulated lipolysis to the nonstimulated lipolysis in the respective strains.

2.4. Cyclic adenosine monophosphate accumulation

One hundred milligrams of the epididymal adipose tissue was cut into small pieces and suspended in 800 μL of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1.15 mmol/L calcium, 3% bovine serum albumin, 10 mmol/L glucose, and 2 mmol/L adenosine triphosphate. After the addition of 100 μL of 1 mmol/L isobutylmethylxanthine, the samples were incubated at 37°C for 10 minutes. One hundred microliters of 1 mmol/L NE or 50 U of ACTH solution was added, and the samples were incubated with shaking at 37°C for 5 minutes. The incubation was stopped by cooling on ice, and 1 mL of 12% trichloroacetic acid was added to the sample. The level of cAMP was measured by radioimmunoassay using the cAMP [125I] Biotrak Assay System (Amersham Biosciences, Buckinghamshire, UK). Cyclic AMP accumulation was represented by the amount of cAMP per DNA.

2.5. Quantitative real-time polymerase chain reaction

Total RNA was extracted from rat adipose tissues using Sepasol RNA I Super (Nacalai Tesque). One microgram of total RNA from each sample was reverse transcribed to complementary DNA (cDNA) using the reverse transcription reagent (Takara, Shiga, Japan) and oligo dT primers (Takara). In a final volume of 20 μL, 2.5 ng of cDNA was mixed with the SYBR Premix Ex Taq reagents (Takara) and the ROX Reference Dye (Takara) to calibrate the fluorescence detection and primers. The primer pairs were selected to yield a single amplicon based on dissociation curves and analysis by agarose gel electrophoresis. The sequences were as follows: β-actin (235 base pairs [bp]), 5′-TGGTCGTACCACTGGCATTG-3′ (forward) and 5′-GAAGTCTAGGGCAACATAGC-3′ (reverse); β_3-adrenergic receptor (adrb3, 268 bp), 5′-TCATCGCCCTGAACTGGTTG-3′ (forward) and 5′-TCCAGAAGTCAGGCTCCTTG-3′ (reverse); melanocortin 2 receptor (mc2r, 150 bp), 5′-TGAAGGGTGCCATGACATTG-3′ (forward) and 5′-TCAAATGCCATT-

Table 2
Release of glycerol stimulated by NE and ACTH in WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats

	Vehicle	NE		ACTH	
		0.01 mmol/L	0.1 mmol/L	0.5 U	5 U
Epididymal					
WKY	1.10 ± 0.13	2.06 ± 0.24**	2.56 ± 0.39**	1.75 ± 0.22**	1.78 ± 0.55**
+/+	0.93 ± 0.21	2.03 ± 0.41**	2.61 ± 0.60**	1.61 ± 0.21**	1.81 ± 0.21**
cp/cp	1.63 ± 0.46	1.81 ± 0.58	2.37 ± 0.33	2.13 ± 0.41	2.29 ± 0.77
Retroperitoneal					
WKY	1.08 ± 0.31	2.10 ± 0.49**	2.51 ± 0.70**	1.18 ± 0.25	1.50 ± 0.38
+/+	1.12 ± 0.47	2.67 ± 1.01**	3.83 ± 1.17**	2.48 ± 0.46*	2.58 ± 0.73**
cp/cp	1.74 ± 0.23	1.95 ± 0.25	2.16 ± 0.31	1.63 ± 0.20	1.86 ± 0.30
Mesenteric					
WKY	0.82 ± 0.14	2.51 ± 0.76**	2.84 ± 0.67**	1.78 ± 0.60*	1.82 ± 0.69*
+/+	0.79 ± 0.07	3.19 ± 0.55**	3.78 ± 0.43**	2.18 ± 0.38**	2.34 ± 0.50**
cp/cp	1.24 ± 0.42	1.86 ± 0.13*	2.28 ± 0.39**	1.58 ± 0.40	1.65 ± 0.23
Subcutaneous					
WKY	0.56 ± 0.19	0.58 ± 0.15	0.59 ± 0.15	0.50 ± 0.16	0.53 ± 0.18
+/+	0.70 ± 0.51	0.99 ± 0.67	1.14 ± 0.74	0.70 ± 0.49	0.74 ± 0.49
cp/cp	1.48 ± 0.40	1.42 ± 0.25	1.73 ± 0.42	1.32 ± 0.28	1.40 ± 0.28

Values are expressed as mean ± SD (n = 4). Glycerol release is represented by the ratio of glycerol (micrograms) to DNA (micrograms).
* $P < .05$.
** $P < .01$ vs vehicle.

GACCTGG-3′ (reverse); hormone-sensitive lipase (hsl, 313 bp), 5′-TGCGCAGGAGTGTGTCTGAG-3′ (forward) and 5′-AGGACACCTTGGCTTGAGCG-3′ (reverse); perilipin (peri, 251 bp), 5′-ACACACCGTGCAGAAGACTC-3′ (forward) and 5′-CGATGTCTTGGAATTCGCTC-3′ (forward). 2Quantitative real-time polymerase chain reaction was performed in the 7000 Real-Time PCR System (Applied Biosystems, Foster City, CA). The thermal cycle programs were as follows: 10 seconds at 95°C followed by amplification of the cDNA for 40 cycles with 5 seconds at 95°C and 32 seconds at 62°C for all primers. A standard curve was run on each plate to validate the method and to determine the efficiency of the reaction, which was taken into account in the calculations. The cDNA for the standard curve was prepared from a mixture of RNA extracted from epididymal, retroperitoneal, mesenteric, and subcutaneous adipose tissues in WKY rats. The concentration of this RNA was determined by spectrophotometry. The standard curve for each gene showed linearity. All samples were run in duplicate, and the values were normalized to the values of β-actin.

2.6. Statistical analysis

All data are presented as mean ± SD. Differences in physiologic parameters, ratios of glycerol release and cAMP accumulation, and messenger RNA (mRNA) expression between each animal were analyzed by 1-way analysis of variance, followed by the Fisher protected least significant difference test. Comparisons of glycerol release and cAMP accumulation stimulated by NE and ACTH with vehicle in the adipose tissues were made by 1-way analysis of variance, followed by the Bonferroni test. Comparisons of glycerol release stimulated by 8-bromo-cAMP with vehicle were made with the Student t test. Statistical significance was set at $P < .05$. Analyses were performed using Statview 5.0 (SAS Institute, Cary, NC) and Excel 2000 (Microsoft, Redmond, WA).

3. Results

3.1. Physiologic parameters and characteristics of adipose tissues in WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats

The body weight and blood pressure of cp/cp rats were higher than those of WKY (Table 1) (body weight, $P < .05$; blood pressure, $P < .05$). The wet weights of the epididymal, retroperitoneal, mesenteric, and subcutaneous adipose tissues of cp/cp rats were higher than the values of +/+ and WKY rats (epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; subcutaneous, $P < .05$). Furthermore, cell sizes were larger in the adipose tissues from cp/cp rats than those from +/+ and WKY rats (epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; subcutaneous, $P < .05$). DNA levels were lower in the adipose tissues from cp/cp rats than those from WKY rats (epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; subcutaneous, $P < .05$). The triacylglyceride contents of mesenteric and subcutaneous adipose tissues from cp/cp rats were higher than those from +/+ and WKY rats (epididymal, $P = .36$; retroperitoneal, $P = .93$; mesenteric, $P < .05$; subcutaneous, $P < .05$).

3.2. Glycerol-releasing activity of NE or ACTH in epididymal, retroperitoneal, mesenteric, and subcutaneous adipose tissues from WKY, +/+, and cp/cp rats

Norepinephrine and ACTH stimulated glycerol-releasing activity in epididymal, retroperitoneal, and mesenteric adipose tissues from WKY and +/+ rats, but not in tissues from cp/cp rats (Table 2) (WKY: epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; +/+: epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; cp/cp: epididymal, $P = .56$; retroperitoneal, $P = .19$; mesenteric, $P < .05$). However, NE and ACTH had no effect on the

Table 3
The ratio of hormone-stimulated glycerol release in various adipose tissues from WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats

	NE		ACTH	
	0.01 mmol/L	0.1 mmol/L	0.5 U	5 U
Epididymal				
WKY	1.90 ± 0.19	2.35 ± 0.28	1.62 ± 0.22	1.63 ± 0.45
+/+	2.22 ± 0.32	2.84 ± 0.35	1.77 ± 0.24	2.00 ± 0.30
cp/cp	1.13 ± 0.31*,†	1.52 ± 0.35*,†	1.37 ± 0.35	1.41 ± 0.30
Retroperitoneal				
WKY	2.00 ± 0.41	2.39 ± 0.56	1.13 ± 0.24	1.41 ± 0.14
+/+	2.48 ± 0.54	3.68 ± 1.07*	2.52 ± 1.16*	2.58 ± 1.16*
cp/cp	1.12 ± 0.07*,†	1.24 ± 0.13*,†	0.94 ± 0.17†	1.08 ± 0.22†
Mesenteric				
WKY	3.11 ± 0.97	3.55 ± 1.00	2.23 ± 0.80	2.25 ± 0.82
+/+	4.02 ± 0.59	4.75 ± 0.15*	2.75 ± 0.40	2.94 ± 0.43
cp/cp	1.60 ± 0.46*,†	1.92 ± 0.39*,†	1.33 ± 0.39*,†	1.42 ± 0.42†
Subcutaneous				
WKY	1.08 ± 0.24	1.09 ± 0.24	0.90 ± 0.13	0.98 ± 0.25
+/+	1.50 ± 0.29*	1.73 ± 0.25*	1.04 ± 0.24	1.12 ± 0.19
cp/cp	0.98 ± 0.11†	1.20 ± 0.30†	0.91 ± 0.09	0.97 ± 0.14

Values are expressed as mean ± SD (n = 4). Each value is represented by the ratio of the hormone-stimulated glycerol release to the nonstimulated glycerol release.

* $P < .05$ vs WKY.

† $P < .05$ vs +/+.

release of glycerol in subcutaneous adipose tissues from WKY, +/+, and cp/cp rats (WKY, $P = 1.89$; +/+, $P = 1.50$; cp/cp, $P = .99$). The ratios of NE-stimulated glycerol release in each adipose tissue from cp/cp rats were lower than those from WKY and +/+ rats, and the ratios of ACTH-stimulated glycerol release in retroperitoneal and mesenteric adipose tissues from cp/cp rats were lower than those from +/+ rats (Table 3) (epididymal: 0.01 mmol/L NE, $P < .05$; 0.1 mmol/L NE, $P < .05$; 0.5 U ACTH, $P = .17$; 5 U ACTH, $P = .12$; retroperitoneal: 0.01 mmol/L NE, $P < .05$; 0.1 mmol/L NE, $P < .05$; 0.5 U ACTH, $P < .05$; 5 U ACTH, $P < .05$; mesenteric: 0.01 mmol/L NE, $P < .05$; 0.1 mmol/L NE, $P < .05$; 0.5 U ACTH, $P < .05$; 5 U ACTH, $P < .05$; subcutaneous: 0.01 mmol/L NE, $P < .05$; 0.1 mmol/L NE, $P < .05$; 0.5 U ACTH, $P = .46$; 5 U ACTH, $P = .50$).

Table 4
Release of glycerol stimulated by 5 mmol/L 8-bromo-cAMP in various adipose tissues from WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats

	Vehicle	5 mmol/L 8-bromo-cAMP	Glycerol release ratio
Epididymal			
WKY	0.63 ± 0.15	4.41 ± 1.28*	7.07 ± 1.98
+/+	0.80 ± 0.35	4.15 ± 0.89*	5.81 ± 2.17
cp/cp	2.22 ± 1.51	3.11 ± 1.67	1.55 ± 0.41†,‡
Retroperitoneal			
WKY	1.11 ± 0.31	3.19 ± 0.43*	3.00 ± 0.85
+/+	1.57 ± 0.34	3.49 ± 0.99*	2.28 ± 0.67
cp/cp	1.82 ± 0.71	1.75 ± 0.40	1.02 ± 0.19†,‡
Mesenteric			
WKY	1.09 ± 0.14	5.15 ± 1.45*	4.82 ± 1.76
+/+	1.18 ± 0.30	5.23 ± 0.79*	4.65 ± 1.31
cp/cp	2.77 ± 0.20	4.12 ± 0.68*	1.48 ± 0.20†,‡
Subcutaneous			
WKY	1.14 ± 0.83	1.86 ± 0.99	1.98 ± 0.96
+/+	1.54 ± 0.64	2.43 ± 1.46	1.66 ± 0.91
cp/cp	1.99 ± 1.64	2.78 ± 1.35	2.06 ± 1.20

Values are expressed as mean ± SD (n = 4). Glycerol release is represented by the ratio of glycerol (micrograms) to DNA (micrograms). Glycerol release ratio is represented by the ratio of the 8-bromo-cAMP–stimulated glycerol release to the nonstimulated glycerol release.

* $P < .05$ vs vehicle.

† $P < .05$ vs WKY.

‡ $P < .05$ vs +/+.

3.3. Glycerol-releasing activity of 8-bromo-cAMP in epididymal, retroperitoneal, mesenteric, and subcutaneous adipose tissues from WKY, +/+, and cp/cp rats

Although 8-bromo-cAMP enhanced glycerol release in epididymal, retroperitoneal, and mesenteric adipose tissues from WKY and +/+ rats, it did not do so in tissues from cp/cp rats (Table 4) ($P < .05$). However, 8-bromo-cAMP had no effect on the release of glycerol in subcutaneous

Table 5
Accumulation of cAMP induced by NE and ACTH in epididymal adipose tissue from WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats

	Vehicle	0.1 mmol/L NE	5 U ACTH
cAMP accumulation			
WKY	12.3 ± 3.0	35.9 ± 1.6*	21.1 ± 2.9*
+/+	9.1 ± 1.6	32.8 ± 4.9*	23.5 ± 5.2*
cp/cp	9.6 ± 1.0	20.1 ± 2.8*	15.5 ± 2.6*
Accumulation ratio			
WKY		3.03 ± 0.63	1.78 ± 0.40
+/+		3.62 ± 0.43	2.56 ± 0.25†
cp/cp		2.10 ± 0.33†,‡	1.61 ± 0.19‡

Values are expressed as mean ± SD (n = 4). Cyclic adenosine monophosphate accumulation is represented by the amount of cAMP (picomoles) per DNA (micrograms). Accumulation ratio is represented by the ratio of the hormone-stimulated cAMP accumulation to the vehicle-induced cAMP accumulation.

* $P < .05$ vs vehicle.

† $P < .05$ vs WKY.

‡ $P < .05$ vs +/+.

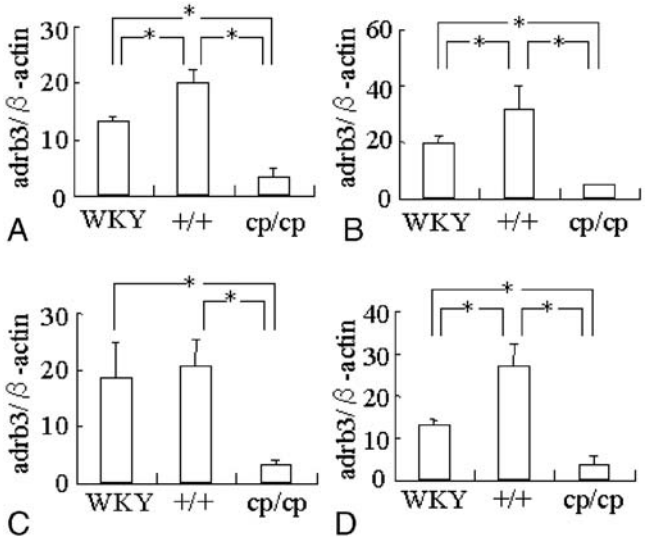

Fig. 2. Differences in the adrb3 mRNA expression in epididymal (A), retroperitoneal (B), mesenteric (C), and subcutaneous (D) adipose tissues from WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats. Values are expressed as mean ± SD (n = 4). *$P < .05$.

adipose tissues from WKY, +/+, and cp/cp rats. The ratios of 8-bromo-cAMP–stimulated glycerol release in these adipose tissues from cp/cp rats were also lower than those from WKY and +/+ rats (epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; subcutaneous, $P = .84$).

3.4. Cyclic adenosine monophosphate accumulation by NE or ACTH in epididymal adipose tissue of WKY, +/+, and cp/cp rats

Norepinephrine and ACTH enhanced the accumulation of cAMP in epididymal adipose tissues from WKY, +/+, and cp/cp rats. However, the rate of cAMP accumulation in cp/cp rats was lower than those in WKY and +/+ rats (Table 5) (cAMP accumulation: WKY, $P < .05$; +/+, $P < .05$; cp/cp, $P < .05$; cAMP accumulation ratio: NE, $P < .05$; ACTH, $P < .05$).

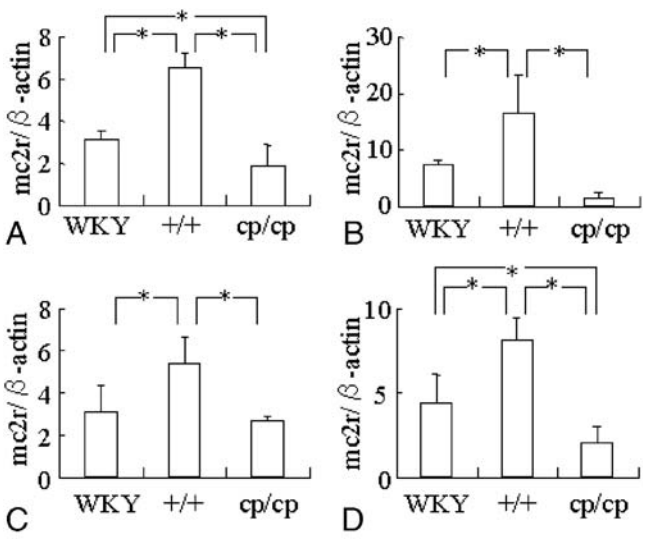

Fig. 3. Differences in the mc2r mRNA expression in epididymal (A), retroperitoneal (B), mesenteric (C), and subcutaneous (D) adipose tissues from WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats. Values are expressed as mean ± SD (n = 4). *$P < .05$.

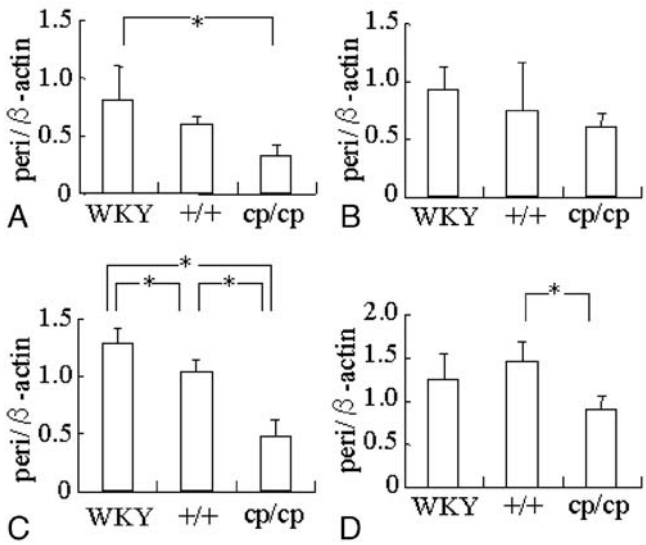

Fig. 4. Differences in the peri mRNA expression in epididymal (A), retroperitoneal (B), mesenteric (C), and subcutaneous (D) adipose tissues from WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats. Values are expressed as mean ± SD (n = 4). *$P < .05$.

3.5. Expression of mRNA for adrb3, mc2r, hsl, and peri in epididymal, retroperitoneal, mesenteric, and subcutaneous adipose tissues from WKY, +/+, and cp/cp rats

We examined differences in the mRNA expression of adrb3 and mc2r, as well as of hsl and peri, in cp/cp rats compared with WKY and +/+ rats. Adrb3 mRNA levels were significantly lower in the adipose tissues from cp/cp

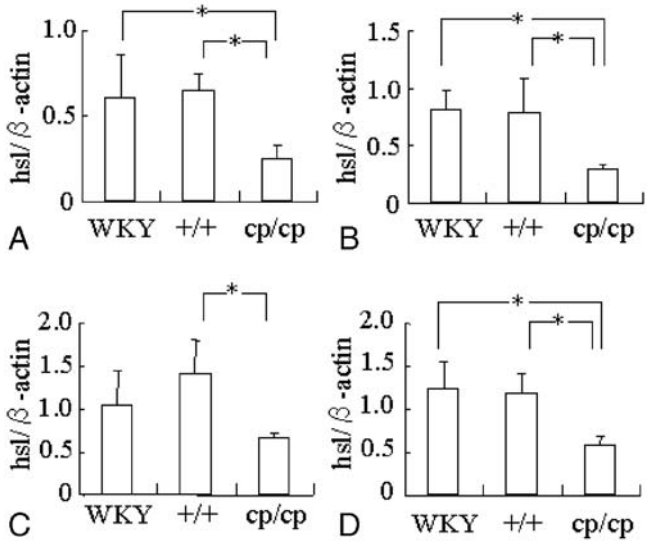

Fig. 5. Differences in the hsl mRNA expression in epididymal (A), retroperitoneal (B), mesenteric (C), and subcutaneous (D) adipose tissues from WKY, SHR/NDmc-cp(+/+), and SHR/NDmc-cp(cp/cp) rats. Values are expressed as mean ± SD (n = 4). *$P < .05$.

rats than those from WKY or +/+ rats (Fig. 2) (epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; subcutaneous, $P < .05$). Similar results were obtained for mc2r mRNA expression in these tissues (Fig. 3) (epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; subcutaneous, $P < .05$). Compared with levels in +/+ rats, hsl mRNA expression in the adipose tissues from cp/cp rats was also significantly decreased, and peri mRNA expression in mesenteric and subcutaneous adipose tissues was also decreased (Figs. 4 and 5) (hsl: epididymal, $P < .05$; retroperitoneal, $P < .05$; mesenteric, $P < .05$; subcutaneous, $P < .05$; peri: epididymal, $P < .05$; retroperitoneal, $P = .32$; mesenteric, $P < .05$; subcutaneous, $P < .05$).

4. Discussion

Corpulent SHR, such as Koletsky, SHR/N-cp, and SHR/NDmc-cp(cp/cp) rats, develop obesity, hypertriglyceridemia, hyperinsulinemia, and hypertension [2-7]. In this article, we ascertained that the cp/cp rats developed hypertrophy of adipocytes as indicated by a large cell size, low DNA content, and high triglyceride content. Among them, we found that DNA contents in subcutaneous adipose tissue from WKY and +/+ rats were larger than those of epididymal, retroperitoneal, and mesenteric tissues and of every tissues from cp/cp rats. These data are consistent with the result from a previous report that the DNA content in subcutaneous tissue was higher than that in the epididymal adipose tissue [20]. The regional differences in DNA content were caused by nonadipocyte DNA contents, which were derived from the stromal-vascular cells [13,19]. Subsequently, it is certain that the current data on DNA content are not completely matched to the number of adipocytes in adipose tissue. Therefore, in the comparisons between various strains of hormone-stimulated glycerol release on the basis of DNA (Table 2), we also performed comparisons of the ratio of the hormone-stimulated lipolysis to the nonstimulated lipolysis in the respective strains (Table 3).

In the tritium incorporation experiment, obese SHR/N-cp rats had higher accumulation of tritiated fatty acids than did lean cohorts [21]. This result suggests that there is a high rate of de novo lipogenesis and/or low rate of lipolysis and fatty acid oxidation in obese SHR/N-cp rats. The current findings are consistent this observation and further showed that cp/cp rats had diminished lipolytic responses to ACTH or NE in epididymal, retroperitoneal, and mesenteric adipose tissues. Furthermore, in comparison with the ratio of the hormone-stimulated lipolysis to the nonstimulated lipolysis, correcting for strain-specific differences in nonadipocyte DNA contents, the lipolytic ratios in cp/cp rats were also lower than those in WKY and +/+ rats. On the other hand, aging was an important factor in lipolytic activity, and blunting lipolytic activity was already developed in rats at 24 weeks of age [22]. We examined NE-stimulated lipolysis in epididymal adipose tissue from WKY and cp/cp rats at 40 weeks of age, and the result indicated that lipolytic response in cp/cp rats was lower than that in WKY rats (data not shown). Accordingly, adipose tissues in mature cp/cp rats were characterized with the low lipolytic activity by a mechanism independent of aging. The weak lipolytic response to ACTH or NE was associated with low levels of mc2r and adrb3 mRNAs, although protein levels were not measured. In the present experiment, the addition of ACTH or NE resulted in less cAMP being accumulated in epididymal adipose tissues of the cp/cp rats, indicating an impairment of receptor-G protein–coupled formation of cAMP. The diminished expression of adrb3 and mc2r mRNAs in adipocytes from cp/cp rats may be due to the so-called insulin resistance associated with marked hyperinsulinemia, as proposed in obese subjects [23,24]. Insulin, which stimulates lipogenesis, in turn, is able to suppress hormone-stimulated accumulation of cAMP, and subsequently impair receptor expression and suppress hormone-sensitive lipase activity.

Norepinephrine-stimulated lipolysis was associated with the activity of hormone-sensitive lipase and hsl mRNA expression in rat visceral fat cells [25]. Decreased hsl expression was demonstrated in abdominal subcutaneous adipocytes of obese subjects [23]. Perilipin, which is a coat protein on the lipid droplets of adipocytes, plays an important role in regulating the hydrolysis of triacylglycerides [26]. In fact, isolated adipocytes from peri null mice, showing a reduction in adipose tissue mass, exhibited elevated basal levels of lipolysis [27]. The decreased mRNA expression for adrb3, mc2r, hsl, and peri may be the chief mechanism of the diminished lipolytic response to ACTH or NE in adipose tissues from the cp/cp rats. Furthermore, the reduced expression of peri mRNA in adipose tissues from cp/cp rats might account for the elevated basal levels of lipolysis in adipose tissues from cp/cp.

It was reported that adipose tissue or adipocytes from SHR, compared with those from normotensive rats, exhibited a strong lipolytic response to ACTH [10] and weak lipolytic response to NE [28-30], respectively. These observations are inconsistent with the present results in +/+ rats. We found that the lipolytic response to hormone and 8-bromo-cAMP stimulation in adipose tissues from +/+ rats did not differ from that in WKY rats, although the levels of adrb3 and mc2r mRNA were higher in the former than in the latter. These results indicated that ACTH- or NE-stimulated lipolysis was the same in adipose tissues from +/+ and WKY rats, but there is a possible genetic predisposition to enhanced lipolysis in +/+ rats. The differences in lipolysis between the previous reports may be due to variations in genetic transmission in SHR rats of different sources [31] and different ages [32].

Norepinephrine and ACTH had no effect on the release of glycerol from subcutaneous adipose tissues of cp/cp rats or WKY and +/+ rats. In addition, the lipolytic response to 8-bromo-cAMP was weak in subcutaneous adipose tissue compared with other visceral adipose tissues such as epididymal, mesenteric, and retroperitoneal adipose tissues. However, the rates of adrb3, mc2r, hsl, and peri mRNA

expression in subcutaneous adipose tissue were the same as those in visceral adipose tissues, in WKY and even in +/+ and cp/cp rats. From these results, it appears that subcutaneous adipose tissue is impaired at the step of post-cAMP signaling for triacylglyceride hydrolysis. A recent study of the regional variation in adipose tissues also showed a difference in numbers of β- or α_2-adrenergic receptors [33-35] and in expression of insulin receptor substrate 1 in subcutaneous tissue from other visceral adipose tissues [8].

Similar as in other tissues, both systems of the sympathetic and parasympathetic neurons are innervated in adipose tissues. In general, the sympathetic nervous system regulates catabolic metabolism [36], whereas the parasympathetic one regulates anabolic metabolism [37]. Very recently, Kreier et al [38] reported that vagal denervation produced the reduction of insulin-mediated glucose and free fatty acid uptake, whereas it increased hormone-sensitive lipase expression. This indicates the involvement of the parasympathetic neuron in lipogenesis in white adipose tissues. In fact, white adipose tissue was suggested to be innervated by the parasympathetic neurons based on pseudorabies virus injection to the local white adipose tissue of sympathectomized rats [38]. Contrary to these studies, Giordano et al [39] have indicated no parasympathetic nerve markers such as vesicular acetylcholine transporter in white adipose tissue of several species and few cells infected with pseudorabies virus from local white adipose tissue in the vagal dorsomotor nucleus. In white adipose tissue of cp/cp rats, however, there are no data dealing with parasympathetic innervation as well as its functional significance, although Kagota et al [40] reported that addition of acetylcholine induced high accumulation of cyclic guanosine monophosphate in aortas of cp/cp rats. Therefore, it is possible that white adipose tissue from cp/cp rats may be already affected by the parasympathetic stimulation, resulting in decreasing hormone-sensitive lipase activity and hormone-stimulated lipolysis response in cp/cp rats judging from high accumulation of triglyceride in white adipose tissue. This surely needs further experiment.

In conclusion, we showed that the lipolysis in adipose tissues from cp/cp rats is markedly diminished because of impairments of pre- and post-cAMP cascades. The impairments would be caused by significant decreases in the mRNA expression of adrb3, mc2r, hsl, and peri in cp/cp rats in spite of the enhanced expression of these genes in +/+ rats.

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