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Metabolism Clinical and Experimental 56 (2007) 1326–1333
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Adrenocortical insufficiency in Otsuka Long-Evans Tokushima Fatty rats, a type 2 diabetes mellitus model

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Received 7 July 2006; accepted 14 May 2007

Abstract

In diabetes, dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis causes effects such as elevation of corticotropin (ACTH) and glucocorticoids. Cholecystokinin and its receptors are involved in the HPA axis and influence the regulation of the HPA axis. We examined adrenocortical function in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of type 2 diabetes mellitus, that lack the cholecystokinin A receptor. We measured adrenal weight, plasma ACTH, serum and urinary corticosterone, and serum leptin in OLETF rats at 5 to 36 weeks of age. Messenger RNA (mRNA) expression of 11β-hydroxysteroid dehydrogenase and 5α-reductase type 1 in adrenal glands of the rats were examined. Long-Evans Tokushima Otsuka (LETO) rats were used as controls. In OLETF rats at 32 to 36 weeks of age, plasma ACTH was significantly higher ($P < .001$); serum corticosterone and 24-hour urinary corticosterone were significantly lower ($P < .005$); and adrenal weight was significantly lower ($P < .005$) than those in LETO rats. At the same ages, serum leptin in OLETF rats was significantly higher ($P < .001$) than that in LETO rats. In the younger OLETF rats, these changes were not observed. Overall, there was an inverse correlation between serum corticosterone and serum leptin ($r = -0.374$, $P < .0005$), whereas there was a positive correlation between plasma ACTH and serum leptin ($r = 0.654$, $P < .0001$). At 5 and 36 weeks of age, mRNA expression of 5α-reductase type 1 in the adrenal gland of OLETF rats was significantly higher ($P < .05$) than that of LETO rats, whereas there was no significant difference in mRNA expressions of 11β-hydroxysteroid dehydrogenase types 1 and 2. We showed that adrenocortical insufficiency and adrenal atrophy were acquired in OLETF rats, and the possibility of elevated serum leptin relates to this phenomenon.

1. Introduction

Dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis such as elevation of corticotropin (ACTH) and glucocorticoids is observed in diabetes of both humans [1,2] and rats [2-4]. In streptozotocin-diabetic rats, as a model of type 1 diabetes mellitus, ACTH and corticosterone are elevated and restored to normal levels by treatment with insulin [4]. This hyperactivation of the pituitary-adrenocortical axis is associated with increased hypothalamic ACTH-releasing hormone messenger RNA (mRNA) [4]. Scribner et al [3] demonstrated that not only ACTH and corticosterone but also adrenal gland weight in streptozotocin-diabetic rats were significantly increased compared to those in the controls.

Otsuka Long-Evans Tokushima Fatty (OLETF) rats were established from Long-Evans rats as a model of type 2 diabetes mellitus in 1992 [5]. The rats have been used as a type 2 diabetes mellitus model in many studies. Otsuka Long-Evans Tokushima Fatty rats have the following characteristic features: (1) late onset of hyperglycemia; (2) a chronic course of disease; (3) mild obesity; (4) inheritance by males; (5) the changes in pancreatic islets such as lymphocyte infiltration and atrophy of the islet, and so on; (6) diabetic nephropathy; and (7) lack of cholecystokinin (CCK)–A receptors [6].

Cholecystokinin stimulates glucocorticoid secretion [7], and activation of CCK-A receptors induces elevation of plasma corticosterone [8]. However, it has been reported that there is no significant difference in ACTH, corticosterone, and adrenal weight between OLETF rats (diabetic) and Long-Evans Tokushima Otsuka (LETO) rats (nondiabetic) [9,10].

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.05.021

In adrenal glands, 11β-hydroxysteroid dehydrogenase (HSD) type 1, 11β-HSD type 2, and 5α-reductase type 1 play important roles in controlling steroid secretion [11-19]. 11β-Hydroxysteroid dehydrogenase catalyzes the interconversion of active glucocorticoids (corticosterone in rodents, cortisol in human) and inactive keto forms (11-dehydrocorticosterone in rodents, cortisone in human). Two isozymes of 11β-HSD, type 1 and type 2, have been identified in humans and rats. In rats, 11β-HSD type 1 activates 11-dehydrocorticosterone into corticosterone, and 11β-HSD type 2 inactivates corticosterone into 11-dehydrocorticosterone. Although being maximally expressed in liver and kidney, 11β-HSD is also present in adrenal glands, especially its type 2 unidirectional isoform [11-13]. 5α-Reductase inactivates corticosterone into their 5α-reduced metabolites. Two isozymes of 5α-reductase, type 1 and type 2, have been identified in humans and rats. The type 1 isozyme is widely distributed throughout the whole body. The type 2 isozyme is preferentially located in male reproductive organs. In the adrenal gland, type 1 mRNA is predominant [18]. There is no report about these enzymes in the adrenal gland of OLETF rats.

In the present study, we tried to clarify the relationship between diabetes and adrenocortical function in OLETF rats by measurement of adrenal weight, serum leptin, plasma ACTH, serum corticosterone, and urinary corticosterone excretion in the long term. We also evaluated the histology of adrenal glands and mRNA expressions of 11β-HSD type 1, 11β-HSD type 2, and 5α-reductase type 1.

2. Materials and methods

2.1. Experimental animals

LETO and OLETF rats at 4 weeks of age were kindly provided by Otsuka Pharmaceuticals (Tokushima, Japan). The rats were bred under the constant environmental conditions of light (12-hour light-dark cycle), room temperature (23°C ± 1°C), and humidity (50% ± 10%). Standard rat chow (CE-2, CLEA Japan, Tokyo, Japan) and water were freely available to the rats. All protocols were approved by the Committee for Animal Research of the Kinki University School of Medicine (Osaka, Japan) and complied with the Principles of Laboratory Animal Care (National Institutes of Health publication no. 85-23, revised 1985).

2.2. Experimental protocol

At 5, 8, 12, 16, 20, 32, and 36 weeks of age, the rats were used for succeeding experiments between 9:00 and 11:00 AM after being fasted for 12 to 16 hours. Peripheral blood was drawn under anesthesia with intraperitoneal injection of pentobarbital (60 mg/kg). Blood levels of glucose, insulin, leptin, ACTH, and corticosterone were measured. Bilateral adrenal glands were removed and weighed in more than 5 rats in each group. At 8 and 32 weeks of age, urinary excretion of corticosterone was evaluated in urine collected over a 24-hour period under food and water intake ad libitum.

2.3. Sample assays

After the blood was drawn, it was separated into 2 tubes, one without anticoagulants for serum and one containing EDTA-dipotassium for plasma, and immediately centrifuged at 3000 rpm for 10 minutes at 4°C, then the supernatant was collected and stored in a deep freezer (−85°C) until assay. Blood glucose was measured by the glucose oxidase method (Kanto Kagaku, Tokyo, Japan). Serum insulin was measured by a radioimmunoassay (RIA) kit (Rat Insulin RIA Kit, Linco Research, St Charles, MO) with a sensitivity of 17.2 pmol/L and a reproducibility of intra-assay variance less than 4.6% and interassay variance less than 9.4% [20]. Serum leptin was measured by an RIA kit (Rat Leptin RIA Kit, Linco Research) with a sensitivity of 30.9 pmol/L and a reproducibility of intra-assay variance less than 4.6% and interassay variance less than 5.7% [20]. Plasma ACTH was measured by an immunoradiometric assay kit (ACTH IRMA, Mitsubishi Chemical, Tokyo, Japan) with a sensitivity of 1.1 pmol/L and a reproducibility of intra-assay variance less than 2.25% and interassay variance less than 0.41% [10]. Serum and urine concentrations of corticosterone were measured by an RIA kit (Coat-A-Count Rat Corticosterone, Diagnostic Products, Los Angeles, CA) with a sensitivity of 58 nmol/L and a reproducibility of intra-assay variance less than 4.0% and interassay variance less than 4.8% [21].

2.4. Histologic evaluation

The adrenal glands were fixed with 10% formaldehyde solution and stained with hematoxylin-eosin. The adrenal tissue was examined histologically under an optical microscope. The thickness of the zona fasciculata was measured at 10 sites of each adrenal gland under a microscope using the Lumina Vision (Mitani Corp, Fukui, Japan) and Mac SCOPE (Mitani Corp) for Macintosh computers. The average thickness of those 10 sites in each adrenal gland was used for analysis.

2.5. RNA extraction and reverse transcription

The removed adrenal glands were minced and homogenized with a sonicator. RNA was extracted with the RNeasy Mini Kit (QIAGEN, Hilden, Germany). Reverse transcription was performed with the High-Capacity complementary DNA (cDNA) Archive Kit (Applied Biosystems, Foster City, CA) in accordance with the manufacturer's instructions (25°C for 10 minutes, 37°C for 120 minutes). The cDNA solution obtained in the reaction was used for polymerase chain reaction (PCR).

2.6. Quantitative real-time PCR

Messenger RNA expressions of 11β-HSD type 1, 11β-HSD type 2, and 5α-reductase type 1 in adrenal glands were quantified by real-time PCR with the ABI PRISM 7700

Sequence Detection System (Applied Biosystems). Real-time PCR was performed on 96-well plates using the Platinum Quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA). In a reaction, the buffer was 25 μL containing Platinum Quantitative PCR SuperMix-UDG 12.5 μL, ROX reference dye 0.5 μL, forward primer (10 μmol/L) 0.5 μL, reverse primer (10 μmol/L) 0.5 μL, fluorogenic probe (10 μmol/L) 0.5 μL, sample template (cDNA generated from 100 ng of total RNA) 5 μL, and autoclaved distilled water 5.5 μL. Oligonucleotide sequences of the primers and probes are shown in Table 1. All target gene probes were labeled with FAM. Oligonucleotide probe of β-actin was labeled with VIC. For PCR, 1 cycle was performed at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The mean data obtained from triplicate PCR in each sample were used for analysis.

In accordance with the manufacturer's guidelines, the data were shown as Ct values, indicating the cycle numbers at which logarithmic PCR plots crossed the threshold line. The relative amount of gene expression was shown as $2^{-\Delta Ct}$, where $\Delta Ct = Ct_{\text{target gene}} - Ct_{\beta\text{-actin}}$.

2.7. Statistical analysis

All results were expressed as mean ± SD. Student *t* test was used to determine the significance between the 2 groups. Correlations were calculated using regression analysis. A *P* value of less than .05 was regarded as significant. Multiple regression analysis was used to examine the association between corticosterone and the other factors.

3. Results

3.1. Body weight, blood glucose levels, and serum insulin levels

There was no significant difference in body weight between LETO and OLETF rats at 5 weeks of age. Otsuka Long-Evans Tokushima Fatty rats were significantly heavier than LETO rats older than 8 weeks (Table 2). There were no significant differences in blood glucose levels between LETO and OLETF rats at 5, 8, and 12 weeks of age. Blood glucose levels in OLETF rats were significantly higher than those in LETO rats older than 16 weeks (Table 2). Serum insulin levels in OLETF rats were significantly higher than those in LETO rats at 5, 12, 16, and 20 weeks of age, but there was no significant difference between LETO and OLETF rats at 8, 32, and 36 weeks of age (Table 2).

Table 1
Oligonucleotide sequences of primers and TaqMan probes

	Primers and probes
11β-HSD type 1	F: ATGGCTTTTGCAGAGCGATT
	R: CAGAGTGGATATCATCGTGGAAGA
	P: FAM-TCTTGGGTGGACTGGACATGCTCATTCT-MGB
11β-HSD type 2	F: AACCTCCAAGGCAGCTATTGC
	R: CGCTTCTCCCAGAGGTTCAC
	P: FAM-TCCAGCCTGGCTGCTTCAAGACAGAG-MGB
5α-reductase type 1	F: GCACTGTTCACACTCAGCACACT
	R: CAACAGCGCTAACAGAGCACTAA
	P: FAM-CAGAGCGAAGCAGCACCATCAGTGG-MGB
β-actin	F: CAGGCATCGCTGACAGGAT
	R: GTGGACAGTGAGGCCAGGAT
	P: VIC-TCAAGATCATTGCTCCTCCTGAGCGC-MGB

Sequences are listed 5′ to 3′. F indicates forward primers; R, reverse primers; P, TaqMan probes.

Table 2
Basic data for LETO and OLETF rats

		Body weight (g)	Glucose (mmol/L)	IRI (pmol/L)
5 wk	LETO	75.8 ± 7.4	5.77 ± 0.94	23.4 ± 14.5
	OLETF	79.3 ± 7.9	6.70 ± 1.44	47.9 ± 32.0 *
8 wk	LETO	210.7 ± 21.8	6.69 ± 0.74	281.4 ± 352.1
	OLETF	242.8 ± 13.9 †	7.52 ± 0.88	391.3 ± 182.5
12 wk	LETO	311.2 ± 26.6	8.20 ± 0.86	427.8 ± 205.4
	OLETF	378.3 ± 30.3 †	9.08 ± 0.66	847.0 ± 355.8 *
16 wk	LETO	411.1 ± 11.8	8.23 ± 1.04	446.6 ± 202.6
	OLETF	532.7 ± 25.0 ‡	10.20 ± 0.82 ‡	783.9 ± 229.4 ‡
20 wk	LETO	445.7 ± 17.9	9.21 ± 1.12	578.4 ± 235.5
	OLETF	565.4 ± 33.2 ‡	11.21 ± 1.14 ‡	1137.1 ± 514.2 ‡
32 wk	LETO	528.8 ± 42.6	8.93 ± 1.14	717.0 ± 402.7
	OLETF	630.8 ± 33.6 ‡	13.33 ± 2.40 ‡	721.1 ± 315.7
36 wk	LETO	527.7 ± 41.5	8.60 ± 1.21	442.2 ± 422.6
	OLETF	608.0 ± 61.2 **	12.91 ± 2.79 ‡	461.7 ± 364.6

Number of LETO and OLETF rats, respectively: 5 weeks = 14 and 9; 8 weeks = 9 and 7; 12 weeks = 6 and 6; 16 weeks = 13 and 12; 20 weeks = 14 and 16; 32 weeks = 13 and 21; 36 weeks = 15 and 15. IRI indicates immunoreactive insulin.

* $P < .05$ compared to LETO rats at the same weeks of age.
** $P < .01$ compared to LETO rats at the same weeks of age.
† $P < .005$ compared to LETO rats at the same weeks of age.
‡ $P < .001$ compared to LETO rats at the same weeks of age.

3.2. Serum leptin levels

There was no significant difference in serum leptin levels between LETO and OLETF rats at 5 weeks of age. Serum leptin levels in OLETF rats were significantly higher than

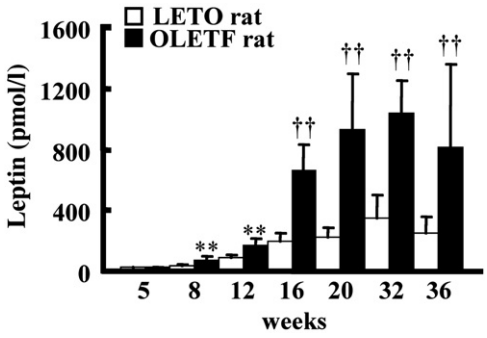

Fig. 1. Serial observation of serum leptin levels in LETO and OLETF rats. Serum leptin levels were significantly higher in OLETF rats older than 8 weeks. Especially, serum leptin levels in OLETF rats were 3- to 4-fold higher than those in LETO rats older than 16 weeks. Number of LETO and OLETF rats, respectively: 5 weeks = 14 and 9; 8 weeks = 9 and 7; 12 weeks = 6 and 6; 16 weeks = 13 and 12; 20 weeks = 14 and 16; 32 weeks = 13 and 21; 36 weeks = 15 and 15. **$P < .01$ and ††$P < .001$ compared to LETO rats at the same age.

those in LETO rats older than 8 weeks. Serum leptin levels in OLETF rats older than 16 weeks were about 3 to 4 times as high as those in LETO rats (Fig. 1).

3.3. Plasma ACTH levels

There was no significant difference in plasma ACTH levels between LETO and OLETF rats at 5, 8, and 12 weeks of age. However, plasma ACTH levels in OLETF rats were significantly higher than those in LETO rats older than 16 weeks (Fig. 2A).

3.4. Serum and urine corticosterone levels

There was no significant difference in serum corticosterone levels between LETO and OLETF rats at 5, 8, and 12 weeks of age. However, serum corticosterone levels in OLETF rats were significantly lower than those in LETO rats older than 16 weeks (Fig. 2B).

There was no significant difference in urinary corticosterone excretion between LETO and OLETF rats at 8 weeks of age (339.1 ± 66.6 nmol/g Cr [n = 9] vs 293.0 ± 74.9 nmol/g Cr [n = 7]). However, urinary corticosterone excretion in OLETF rats was significantly lower than that in LETO rats at 32 weeks of age (311.6 ± 64.7 nmol/g Cr [n = 8] vs 223.8 ± 32.2 nmol/g Cr [n = 8], $P < .005$).

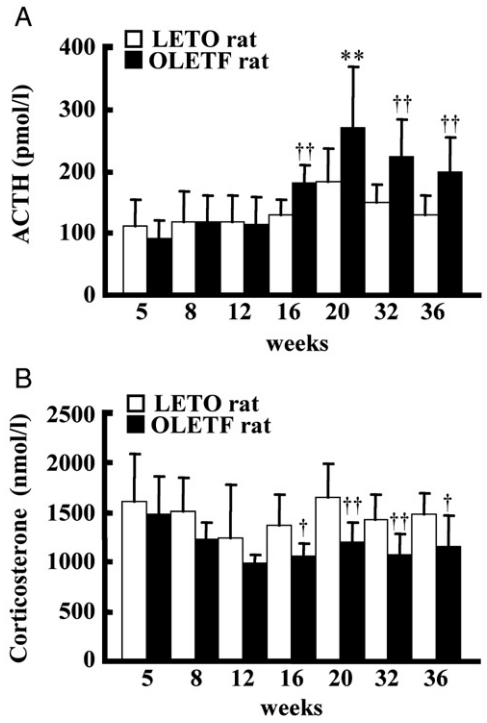

Fig. 2. Comparison of plasma ACTH levels (A) and serum corticosterone levels (B) between LETO and OLETF rats. After 16 weeks of age, plasma ACTH levels in OLETF rats were significantly higher (A) and serum corticosterone levels in OLETF rats were significantly lower (B) than those in LETO rats. Number of LETO and OLETF rats, respectively: 5 weeks = 14 and 9; 8 weeks = 9 and 7; 12 weeks = 6 and 6; 16 weeks = 13 and 12; 20 weeks = 14 and 16; 32 weeks = 13 and 21; 36 weeks = 15 and 15. $^{**}P < .01$, $^{\dagger}P < .005$, and $^{\dagger\dagger}P < .001$ compared to LETO rats at the same age.

3.5. Adrenal weight

Adrenal weights of OLETF rats were significantly lighter than those of LETO rats at 32 and 36 weeks of age, whereas there were no significant differences in adrenal weights between LETO and OLETF rats at 5, 8, 12, 16, and 20 weeks of age (Fig. 3A).

3.6. Histologic examination

The typical histology of adrenal glands of a LETO rat and an OLETF rat at 36 weeks of age is shown in Fig. 3B1 and 3B2. The zona fasciculata of the adrenal gland of the OLETF rat was obviously thinner than that of the LETO rat. The thickness of the zona fasciculata of adrenal glands of OLETF rats was significantly thinner than that of LETO rats at 36 weeks of age (LETO rats [n = 5] vs OLETF rats [n = 5]: 429.2 ± 32.6 vs 326.8 ± 24.9 μm, $P < .002$). However, there was not any other abnormal finding such as bleeding, inflammation, or necrosis in the adrenal glands of OLETF rats.

3.7. Correlations between adrenal function and the other parameters

Correlations between adrenal function (ACTH, corticosterone, and adrenal weight) and other parameters (glucose, insulin, and leptin) were evaluated.

In LETO rats (n = 84), plasma ACTH levels showed significant positive correlations with blood glucose levels ($r = 0.397$, $P < .0005$), serum insulin levels ($r = 0.327$, $P < .005$), and serum leptin levels ($r = 0.341$, $P < .005$). In OLETF rats (n = 86), plasma ACTH levels showed significant positive correlations with blood glucose levels ($r = 0.412$, $P < .0001$), serum insulin levels ($r = 0.551$, $P < .0001$), and serum leptin levels ($r = 0.654$, $P < .0001$). Plasma ACTH levels in OLETF rats showed stronger positive correlations with all the parameters than those in LETO rats. In OLETF rats, plasma ACTH levels showed the strongest positive correlation with serum leptin levels.

Serum corticosterone levels in LETO rats (n = 84) did not correlate with any other parameter (blood glucose, serum insulin, and serum leptin levels), whereas serum corticosterone levels in OLETF rats (n = 86) inversely correlated with blood glucose levels ($r = -0.357$, $P < .001$), serum insulin levels ($r = -0.362$, $P < .001$), and serum leptin levels ($r = -0.374$, $P \leq .0005$). In OLETF rats, serum corticosterone levels showed the strongest inverse correlation with serum leptin levels. Relationships between serum leptin levels and plasma ACTH levels or serum corticosterone levels in each rat strain are shown in Fig. 4.

Adrenal weight in OLETF rats showed a significant inverse correlation with serum leptin levels (Fig. 5), whereas adrenal weight in LETO rats showed a significant positive correlation with serum leptin levels ($r = 0.439$, $P < .005$, n = 70). As shown in Fig. 3A, adrenal weights of OLETF rats showed significant increases between 5 and 8 weeks of age ($P < .01$), and there was no significant change between 8 and 12 weeks of age. There were no

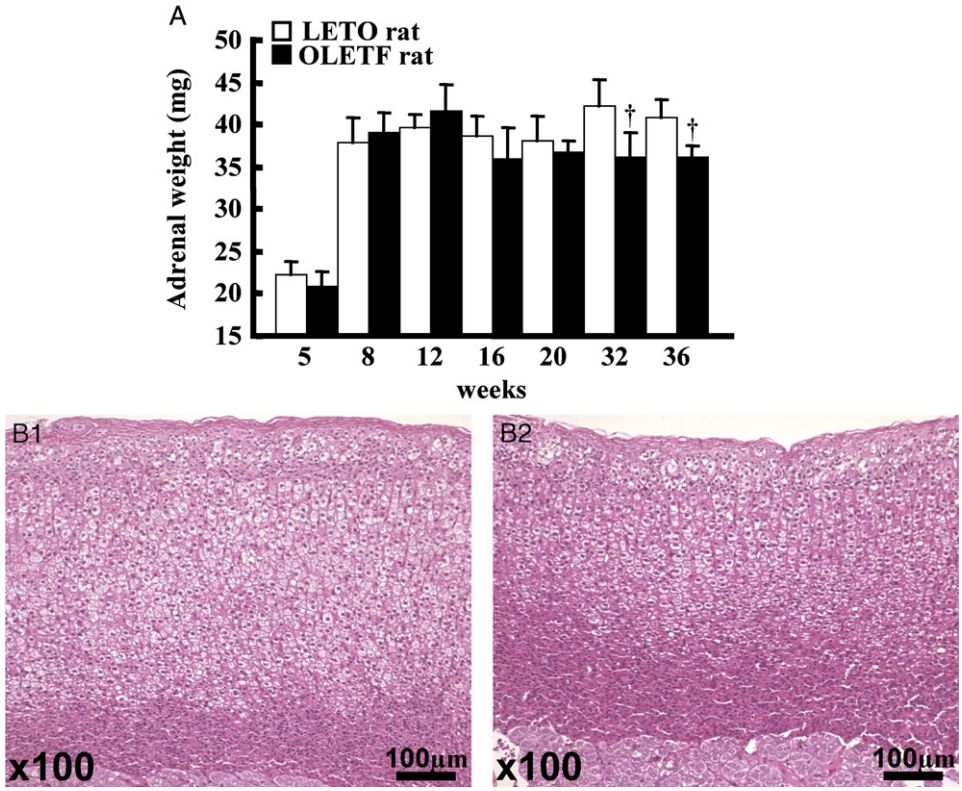

Fig. 3. Adrenal weight (A) and histology of adrenal glands of a LETO rat (B1) and an OLETF rat (B2). After 32 weeks of age, adrenal weights of OLETF rats were significantly lower than those of LETO rats. Number of LETO and OLETF rats, respectively: 5 weeks = 14 and 9; 8 weeks = 9 and 7; 12 weeks = 6 and 6; 16 weeks = 6 and 5; 20 weeks = 6 and 5; 32 weeks = 9 and 17; 36 weeks = 5 and 5. $^{\dagger}P < .005$ compared to LETO rats at the same weeks of age. Typical histology of adrenal glands of a LETO rat and an OLETF rat at 36 weeks of age were shown (hematoxylin-eosin stain). The thickness of the zona fasciculata in the OLETF rat was obviously thinner than that in the LETO rat (scale bar = 100 μm).

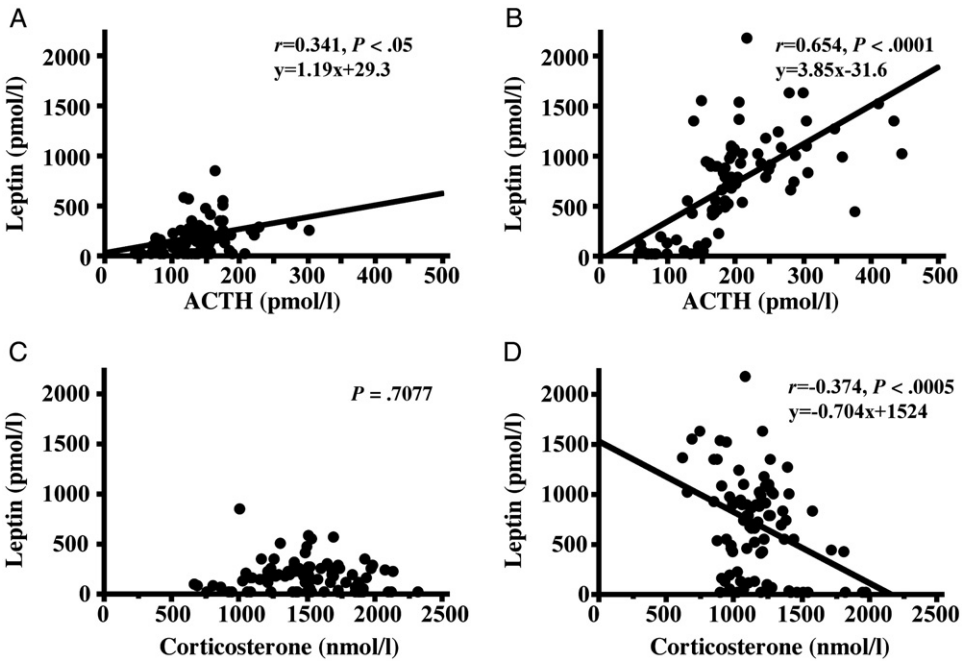

Fig. 4. Correlation between serum leptin levels and plasma ACTH levels in LETO rats (A) and OLETF rats (B), and between serum leptin levels and serum corticosterone levels in LETO rats (C) and OLETF rats (D). A significant positive correlation between serum leptin levels and plasma ACTH levels was observed in both LETO rats (n = 84) and OLETF rats (n = 86), but there was a stronger positive correlation in OLETF rats. A significant inverse correlation between serum leptin levels and serum corticosterone levels was observed in OLETF rats (n = 86) but not in LETO rats (n = 84).

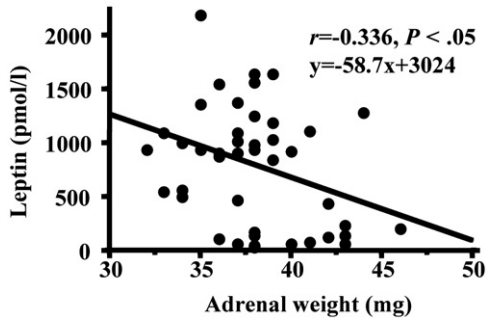

Fig. 5. Correlation between serum leptin levels and adrenal weights of OLETF rats. Adrenal weights of OLETF rats older than 8 weeks were used to calculate correlations between serum leptin levels and adrenal weight. A significant inverse correlation between serum leptin levels and adrenal weights was observed in OLETF rats (n = 45).

significant changes in the adrenal weights of OLETF rats older than 16 weeks. Therefore, the adrenal weights of rats older than 8 weeks were used to calculate correlations between serum leptin levels and adrenal weights. In contrast, adrenal weights of LETO rats showed a significant increase between 5 and 8 weeks of age ($P < .01$) and between 20 and 32 weeks of age ($P < .05$). Because there was no significant change between 32 and 36 weeks of age, the growth of adrenal gland was considered to reach a peak at 32 to 36 weeks of age. Adrenal weights of LETO rats older than 8 weeks were also used for the analysis of correlations between serum leptin levels and adrenal weight.

To clarify what was the most effective factor on corticosterone levels, we performed multiple regression analysis using leptin, insulin, and glucose as independent variables. This multiple regression analysis showed a significant association ($F < .001$) with the serum corticosterone levels. The standardized regression coefficients were −2.39 for leptin, −0.29 for insulin, and −0.31 for glucose, suggesting that leptin was closely associated with the serum corticosterone levels.

3.8. Messenger RNA expressions of 11β-HSDs and 5α-reductase type 1 in the adrenal gland

Messenger RNA expressions of 11β-HSD types 1 and 2 in the adrenal gland did not show significant differences between LETO and OLETF rats at either 5 or 36 weeks of age (Fig. 6A and B). At both 5 and 36 weeks of age, mRNA expression of 11β-HSD type 2 was significantly more than that of 11β-HSD type 1 in both strains of rats. Messenger RNA levels ($2^{-\Delta Ct}$) of 11β-HSD types 1 and 2 were 0.005 (mean) vs 0.07 ($P < .0001$) in 5-week-old LETO rats and 0.006 vs 0.05 ($P < .0001$) in 5-week-old OLETF rats, respectively. This finding suggests that mRNA expression of 11β-HSD type 2 is nearly 10 times higher than that of 11β-HSD type 1 in adrenal glands from both LETO and OLETF rats.

The mRNA expression of 5α-reductase type 1 in OLETF rats was significantly higher than that in LETO rats at both 5 and 36 weeks of age (Fig. 6C).

4. Discussion

In the present study, we showed that primary adrenocortical dysfunction existed in OLETF rats older than 16 weeks. Pentobarbital was used in the present study because it rarely affects blood levels of glucose, insulin, ACTH, or corticosterone [22,23]. The reduction of corticosterone secretion was confirmed by measuring corticosterone in urine collected over a 24-hour period without anesthesia. In addition, the adrenal weights of OLETF rats were significantly lower than those of LETO rats older than 32 weeks. Corticosterone is primarily synthesized in the

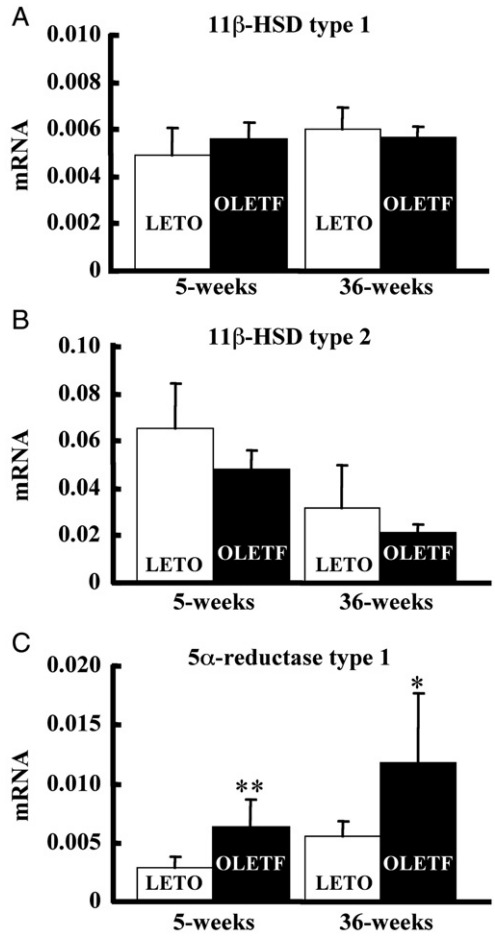

Fig. 6. Messenger RNA expressions of 11β-HSD type 1 (A), 11β-HSD type 2 (B), and 5α-reductase type 1 (C) in the adrenal glands of LETO rats (□) and OLETF rats (■). Messenger RNA expression is shown as a relative amount compared with β-actin mRNA ($2^{-\Delta Ct}$ value). There were no significant differences in mRNA expressions of 11β-HSD types 1 and 2 between LETO and OLETF rats. In OLETF rats, 5α-reductase type 1 mRNA expression was significantly higher than that in LETO rats. Number of LETO and OLETF rats, respectively: 5 weeks = 6 and 6; 36 weeks = 5 and 5. *$P < .05$ and **$P < .01$ compared to LETO rats at the same age.

zona fasciculata in the adrenal cortex. We confirmed that the thickness of zona fasciculata in OLETF rats was significantly reduced compared with that in LETO rats at 36 weeks of age. Thus, these data suggest a reduced ability to synthesize corticosterone in OLETF rats.

Previous studies showed there were no significant differences in adrenal weight and blood corticosterone levels between LETO and OLETF rats [9,10]. However, Ohta et al [9] demonstrated that there was no significant difference in either adrenal weight or blood corticosterone levels between LETO and OLETF rats at 5 and 8 weeks of age, consistent with our findings. Takao et al [10] showed a slight, but not significant, decrease in the adrenal weights of OLETF rats compared with those in LETO rats. In their report, neither the experiment time nor the presence or absence of fasting was described, whereas our experiment was done between 9:00 and 11:00 AM after fasting for 12 to 16 hours. The advantages of our study compared with their report are as follows: (1) larger number of rats with the wider range of age (5-36 weeks of age) were used in our study, whereas their data were obtained from only 6 rats at 30 to 38 weeks of age; (2) plasma glucose levels in OLETF rats were 13.3 ± 2.4 nmol/L (239.9 ± 43.2 mg/dL) at 32 weeks of age in our study, whereas those in their study were 175.4 ± 9.6 mg/dL, indicating diabetes of the rats was more obvious in our study; (3) the reduction of urinary corticosterone excretion was confirmed in our study; and (4) the decreasing thickness of zona fasciculata of the adrenal glands in OLETF rats was confirmed in our study. These differences in our study provided more precise results.

Primary adrenocortical insufficiency in the present study may be caused by the specific natures of OLETF rats such as lack of CCK-A receptor deficiency.

Because CCK stimulates glucocorticoid secretion [7] and the CCK receptor antagonist inhibits elevation of plasma corticosterone [8], the deficiency of CCK-A receptor per se may be a basal or permissive factor to induce adrenal insufficiency.

According to a recent report by Cano et al [24], serum leptin levels were elevated 2- to 2.5-fold after administration of CCK receptor antagonists in rats. In the present study, serum leptin levels in OLETF rats were 3 to 4 times as high as those in LETO rats older than 16 weeks. It is considered that hyperleptinemia due to obesity is further enhanced by CCK-A receptor deficiency. In the adrenal gland, leptin inhibits glucocorticoid secretion from bovine [25], rat [26], and human [26,27] adrenocortical cells in vitro. In vivo, serum corticosterone is decreased by leptin administration in rats [28]. Furthermore, leptin reduces the volume of the zona fasciculata, resulting in adrenal atrophy [29]. In our study, both serum corticosterone levels and adrenal weights showed inverse correlations with serum leptin levels in OLETF rats. Adrenocortical dysfunction of OLETF rats in this study may be caused by the hyperleptinemia enhanced with the CCK-A receptor deficiency. Indeed, multiple regression analysis using leptin, insulin, and glucose levels as independent variables showed a significant association with the serum corticosterone levels, and the standardized regression coefficient of leptin showed the highest value. It is possible that the other factors, not measured in this study, influence the adrenocortical dysfunction in OLETF rats. However, we consider that leptin is at least one factor underlying the reduced adrenal function in OLETF rats.

There was no significant difference in mRNA expression of 11β-HSDs (types 1 and 2) between LETO and OLETF rats. This indicated that these enzymes did not influence the reduction of corticosterone secretion in OLETF rats. Meanwhile, mRNA expression of 5α-reductase type 1 in OLETF rats was significantly higher than that in LETO rats at both 5 and 36 weeks of age. It is possible that the mRNA expression of 5α-reductase type 1 in the adrenal gland of OLETF rats is enhanced either congenitally or from an early age. This is the first report about increased mRNA expression of 5α-reductase type 1 in the adrenal gland of OLETF rats. The enhanced expression of 5α-reductase type 1 may contribute to the reduction of corticosterone secretion in OLETF rats. Interestingly, mRNA expression of 5α-reductase type 1 was increased at an older age in both LETO and OLETF rats. This enzyme may be increased with aging. It is unclear how increased 5α-reductase type 1 in OLETF rats affects diabetes. Further investigation is needed to elucidate the role of 5α-reductase type 1 in OLETF rats.

Many reports demonstrated that serum cortisol levels were elevated in human type 2 diabetes mellitus [1,2]. The mechanisms of increment of cortisol are not known yet. However, in the recent reports, chronic hyperglycemia in diabetic patients is associated with a defect in the glucocorticoid receptor function that may include negative feedback by cortisol in the central nervous system and pituitary [30,31]. So far, glucocorticoid is generally increased in human type 2 diabetes mellitus, whereas corticosterone in OLETF rats is decreased in the present study. Therefore, the changes in ACTH and corticosterone in the OLETF rats were opposite to those in human diabetes. These differences in adrenocortical function might be caused by the specific nature of OLETF rats including CCK-A receptor deficiency and hyperleptinemia as described above.

In conclusion, we demonstrated that acquired adrenocortical insufficiency existed in OLETF rats, which might be associated with hyperleptinemia. These new findings are interesting to consider in the relationship among diabetes, the HPA axis, and leptin. The present study may provide new insight into the pathophysiology of type 2 diabetes mellitus and obesity.

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