［原著］Supplementary date for "Cholecystokinin was involved in the development of leptin resistance in OLETF rats"

Supplementary data for "Cholecystokinin was involved in the development of leptin resistance in OLETF rats" [Ryukyu Med. J., 27(3, 4) 105-114, 2008]

Makoto Motomura, Masanori Sunagawa, Mariko Nakamura and Tadayoshi Kosugi

1st Department of Physiology, Unit of Physiological Science, School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan

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As a result of continued interest and the following questions, additional data and explanations are provided in this supplement.

1. Is there a possibility that it is not leptin resistance but rather the lack of CCK1 receptors itself that is the main cause for obesity in OLETF rats?

OLETF rats lack CCK1 receptors (CCK1R) and spontaneously exhibit the onset of non-insulin-dependent diabetes mellitus and mild obesity through their overeating behavior. The mRNA expression of CCK1R in the hypothalamus was measured by reverse transcriptase polymerase chain reaction (RT-PCR). As shown in Fig. S 1, the OLETF rats lacked CCK1R expression in the hypothalamus. Cloning and sequencing of the CCK1R gene in the OLETF rats identified a 6847 bp. - deletion in its promoter region and the first and second exons. However, the OLETF rats were inbred through natural mating, and whether hyperphagia-induced obesity is caused solely by the deficiency of CCK1R is not determined. It is not verified whether the lack of CCK1R is the only genetic abnormality that leads to leptin resistance in OLETF rats. There is no report that obesity is observed in CCK1R knockout mice. Therefore, other inborn genetic abnormalities might be involved in leptin resistance in OLETF rats.

2. How about comparison of the leptin resistance index (LRI) between LETO and OLETF rats at the age of 7 weeks?

We reported that the leptin resistance index (LRI) was significantly increased in OLETF rats at the age of 38 weeks. In addition, the LRI was

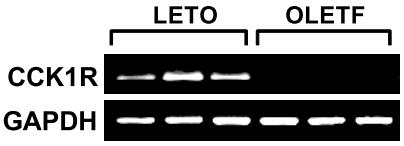

Fig. S1 Expression of CCK1 receptors (CCKIR) mRNA in the hypothalamus
Total RNA was extracted from hypothalamic tissues of three different LETO and OLETF rats. After reverse transcription, PCR was performed using the primers for rat CCK1R and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR products were separated on 1.7% agarose gel containing ethidium bromide with 44.5 mM Tris-borate/1mM EDTA electrophoresis buffers (pH 7.5).

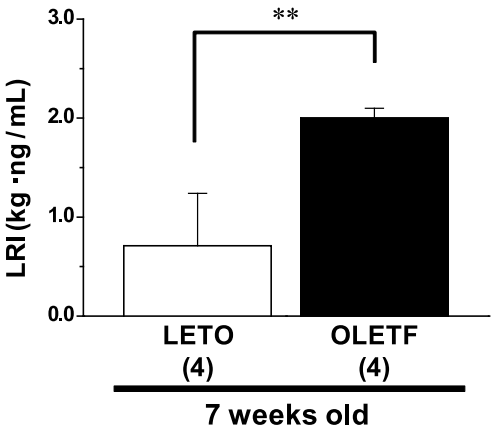

Fig. S2 Comparison of the leptin resistance index (LRI) of LETO and OLETF rats
The mean values of LRI in the LETO (open bar) and OLETF (closed bar) rats were calculated from data at seven weeks old. The data represent mean ± SE. The numbers in parentheses represent the number of rats tested. ** indicates a statistically significant difference with $P<0.01$, as compared to age-matched LETO rats using Student's unpaired *t*-test

significantly increased in OLETF rats even at the age of seven weeks (Fig. S 2). It suggested that leptin resistance manifested from an early age in the OLETF rats.

3. Is there a possibility that it is not leptin resistance but rather an elevated level of active ghrelin that is the main cause for obesity in OLETF rats?

We examined whether or not obesity in OLETF rats is caused by elevated level of active ghrelin. To do this, we collected data from the five LETO rats with the highest levels of active ghrelin in the group and also collected data from the five OLETF rats with the lowest levels of active ghrelin in the group. The mean value of active ghrelin in the OLETF rats was lower than that of the LETO rats (Table S 1) by this grouping. Consequently, body weight was still higher in OLETF than LETO rats. Thus, obesity could be induced not only by elevated active ghrelin but also by leptin resistance in OLETF rats.

Table S1 Comparison of active ghrelin and body weight at the age of 38 weeks in LETO and OLETF rats

	Rats	Active ghrelin		Body weight	
		(fmol/ml)	ratio*	(g)	ratio*
All data	LETO (16)	11.2 ± 1.8	1.74	495.3 ± 7.7	1.37
	OLETF (12)	19.6 ± 2.6		680.8 ± 14.5	
Selected data**	LETO (5)	19.3 ± 3.0	0.82	486.0 ± 12.1	1.44
	OLETF (5)	15.8 ± 2.0		702.0 ± 21.1	

* Ratios are calculated by dividing the mean value of OLETF rats by that of LETO rats
** Data from the LETO rats with the five highest values of active ghrelin and from the OLETF rats with the five lowest values are compared.