

# Cardiac BDNF/TrkB Signaling Can Be Induced By Calorie Restriction With Improved Physical Activity In Obese Mice

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## Abstract

Central nervous system mediates cardiac dysfunction. Brain-derived neurotrophic factor (BDNF) gene is expressed not only in neurons but in cardiac muscle, but cardioprotective effect of BDNF is not yet clear. We hypothesized that cardiac signaling of BDNF/tyrosine kinase receptor B (TrkB) could be induced by the restriction of food in obese subjects, and that induced BDNF signaling could be beneficial in cardiac dysfunction. KKAY mice were fed ad libitum as control (KKAY), or 60% of ad libitum levels (CR-KKAY). Mice were sacrificed on day 0, 7, 28, and 84 of caloric restriction. Body weight and liver/body weight ratio in CR-KKAY were significantly lower than in KKAY on day 7, 28 and 84. However, heart/body weight ratio did not differ between KKAY and CR-KKAY. Cardiac BDNF mRNA level in CR-KKAY was significantly higher than that in KKAY ( $1.2 \pm 0.3$  vs.  $0.8 \pm 0.2$ ,  $P < 0.05$ ). Cardiac expression of TrkB in CR-KKAY was significantly lower than that in KKAY on day 28. Hippocampal expression of BDNF in CR-KKAY was significantly higher compared with KKAY on day 28 and 84. Hippocampal weight/body weight ratio was also significantly elevated in CR-KKAY compared with KKAY on day 28 and 84. Physical activity in CR-KKAY was significantly increased compared with that in KKAY on day 84.

Cardiac expression of BDNF was induced by caloric restriction with the increase of physical activity in obese mice. Cardiac BDNF may play a role in recovery from obese related dysfunction of the heart. We demonstrate that induced expression of BDNF/TrkB signaling can improve heart failure in obese mammals with the combination of increased hippocampal expression of BDNF.

## INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of neurotrophic factors, has important functions in the peripheral and central nervous system of vertebrates [1]. BDNF was originally isolated from the brain; however, it is also expressed in the non-neural tissues [2]. BDNF is expressed in cardiac muscle and may modulate cardiac function. Caloric restriction (CR) is beneficial for myocardial ischemia through the BDNF signaling pathway [3]. Moreover, CR improves heart failure in obese subjects [4] and is closely associated with the increase of hippocampal BDNF mRNA [5]. However, the relation between BDNF and heart disease is not yet clear [6]. We hypothesized that cardiac and hippocampal expression of BDNF could be induced by the restriction of food in obese subjects and that induced BDNF signaling could be beneficial in cardiac dysfunction.

## METHODS

### Mice and experimental protocol

Obese KKAY mice were fed ad libitum as control (KKAY,  $n=18$ ) or 60% restriction of ad libitum levels (CR-KKAY,  $n=18$ ). As a normal control, C57BL mice were fed ad libitum (C57BL,  $n=18$ ). Mice were sacrificed on day 0, 7, 28, and 84 after food restriction. Cages were maintained under a light-dark photoperiod (lights on from 9 AM to 5 PM vs. lights off from 5 PM to 9 AM) provided by fluorescent bulbs installed under the ceiling. Environmental air temperature was maintained at between 24 and 25°C. Approval for this experiment was obtained from the animal experiment committee at Kanazawa Medical University.

### Daily activity

Voluntary movement during day and night time was analyzed by ACTOMO-10 animal activity monitors (Moter Activity Measurement System, Shintech, Fukuoka), which continuously compiles data [7]. In brief, movement is

allowed within a rectangular enclosure (30x20 cm<sup>2</sup>) with a side wall equipped with photosensors at 2-cm intervals, as described previously. Total activity counts in each 10-min segment were automatically recorded for 30 min. Results from monitoring were used to determine whether CR might have affected the voluntary movement during the day and night time.

**Real-time PCR**

The heart and brain were rapidly isolated on an ice-cold platform, then frozen in liquid nitrogen and stored in a -80°C refrigerator until analysis of the related gene expression described previously [8]. RNA extraction from frozen heart and hippocampus tissue was performed as described by the manufacturer (RNeasy Mini Kit; QIAGEN, Tokyo, Japan). BDNF and TrkB-PCR were Mm00432069\_m1 and Mm00432069\_m1, respectively (Applied Biosystems). Quantification of target cDNA (BDNF, TrkB) and an internal reference gene (GAPDH) were performed in 96-well plates on the ABI PRISM7700 Sequence Detection System, and analyses were performed using machine software. Each sample was analyzed in triplicate.

**Statistical Analysis**

Data are expressed as mean ± SD. The BW and heart/body weight ratio, hippocampal weight/body weight ratio, liver weight/body weight ratio were compared by Student’s t test between model mice and normal controls. The voluntary movements in KKAY mice were compared with the control using Student’s t test. The expression levels of BDNF and TrkB mRNA were also evaluated by one-way ANOVA.

**RESULTS**

Body weights in CR-KKAY were significantly lower than those in KKAY on day 7, 28, and 84. The body weights in both KKAY and CR-KKAY were significantly higher than those in C57BL on day 0, 7, 28, and 84 after treatment. Heart/ body weight ratio were not significantly different between CR-KKAY and KKAY; however, those in CR-KKAY and KKAY were significantly higher than in C57BL on day 0, 7, 28 and 84. Right hippocampal/body weight in CR-KKAY was significantly higher than in KKAY on day 28 and 84. The ratio in both KKAY and CR-KKAY was significantly lower than in C57BL on day 0, 7, 28, and 84 after treatment. Liver / body weight ratio in CR-KKAY was significantly lower than in KKAY on day 7, 28 and 84 (P<0.05); however, there was no difference among the three groups on day 0. (Table 1)

**Table 1**  
Results

	C57BL	KKAY	CR-KKAY
<b>Day 0</b>			
BW (g)	19.2 ± 0.5	40.3 ± 0.8*	40.4 ± 0.8*
HW (mg)	94 ± 1.8	151 ± 2.8*	153 ± 3.4*
HW/BW ratio (10 <sup>-2</sup> )	48.2 ± 2.2	38.6 ± 2.1*	38.8 ± 2.4*
Hippocampal weight (mg)	1.77 ± 0.42	0.99 ± 0.23*	0.97 ± 0.25*
Hippocampal weight/BW ratio (10 <sup>-5</sup> )	9.2 ± 0.44	2.5 ± 0.32*	2.4 ± 0.27*
Liver weight (mg)	998 ± 122	2015 ± 234*	1980 ± 197*
Liver weight/BW ratio (10 <sup>-2</sup> )	51.1 ± 4.3	48.9 ± 4.5*	47.8 ± 4.4*
<b>Day 7</b>			
BW (g)	19.5 ± 0.6	41.9 ± 0.9*	38.1 ± 0.8**
HW (mg)	94 ± 1.8	151 ± 2.8*	153 ± 3.4*
HW/BW ratio (10 <sup>-2</sup> )	48.2 ± 2.2	38.9 ± 2.0*	39.2 ± 1.7*
Hippocampal weight (mg)	1.75 ± 0.40	0.81 ± 0.27*	0.95 ± 0.22*
Hippocampal weight/BW ratio (10 <sup>-2</sup> )	9.1 ± 0.42	4.3 ± 0.39*	4.8 ± 0.21*
Liver weight (mg)	1125 ± 103	2145 ± 234*	1452 ± 167**
Liver weight/BW ratio (10 <sup>-2</sup> )	57.7 ± 4.1	51.2 ± 7.2*	38.1 ± 4.0**
<b>Day 28</b>			
BW (g)	20.6 ± 0.8	45.6 ± 1.3*	34.4 ± 1.0**
HW (mg)	94 ± 1.8	151 ± 2.8*	153 ± 3.4*
HW/BW ratio (10 <sup>-2</sup> )	48.2 ± 2.2	38.6 ± 2.1*	38.8 ± 2.4*
Hippocampal weight (mg)	1.81 ± 0.43	0.99 ± 0.23*	0.97 ± 0.25*
Hippocampal weight/BW ratio (10 <sup>-2</sup> )	8.8 ± 0.44	2.2 ± 0.34*	2.8 ± 0.30*
Liver weight (mg)	998 ± 122	2015 ± 234*	1980 ± 197*
Liver weight/BW ratio (10 <sup>-2</sup> )	51.1 ± 4.3	48.9 ± 4.5*	47.8 ± 4.4*
<b>Day 84</b>			
BW (g)	21.8 ± 1.3	50.2 ± 2.0*	33.5 ± 1.7**
HW (mg)	108 ± 2.9	163 ± 3.6*	114 ± 3.2**
HW/BW ratio (10 <sup>-2</sup> )	49.4 ± 3.3	32.6 ± 2.3*	34.1 ± 2.1*
Hippocampal weight (mg)	1.77 ± 0.42	1.12 ± 0.23*	1.07 ± 0.28*
Hippocampal weight/BW ratio (10 <sup>-2</sup> )	9.23 ± 0.44	2.21 ± 0.32*	3.19 ± 0.37**
Liver weight (mg)	1290 ± 183	2680 ± 234*	1165 ± 167**
Liver weight/BW ratio (10 <sup>-2</sup> )	59.2 ± 7.3	53.4 ± 4.9*	34.8 ± 4.0**

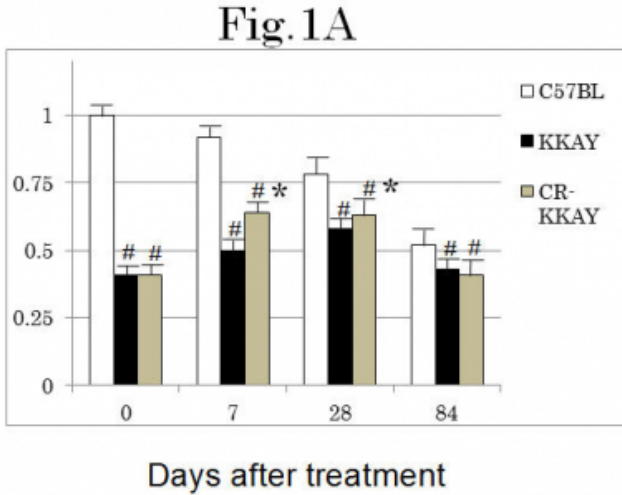
Abbreviations; HW; heart weight, BW; body weight, \*P<0.05 vs. C57BL mice, \*\*P<0.05 vs. KKAY mice.

Comparative expression of cardiac BDNF mRNA in KKAY was significantly lower than in C57BL on day 0 and 7. On day 28 and 84, values were not significantly different among the three groups. Cardiac expression of TrkB in CR-KKAY was significantly lower than in KKAY on day 28; however, that in CR-KKAY did not differ from KKAY on day 7 and 84. That in KKAY mice was significantly lower than in C57BL on day 0 and 7 (Fig. 1).

**Figure 1A**

Cardiac expression of BDNF (Fig.1A) by caloric restriction on KKAY obese mice.

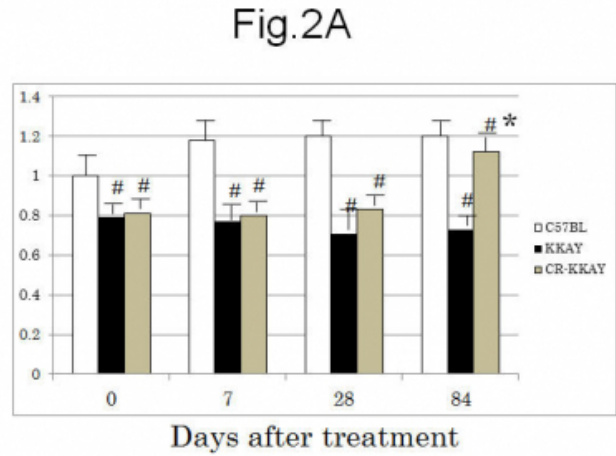
#P<0.05 vs.C57BL wild type mice, \*P<0.05 vs. KKAY obese mice ad libitum.



**Figure 2A**

Hippocampal expression of BDNF (Fig.2A) by caloric restriction on KKAY obese mice.

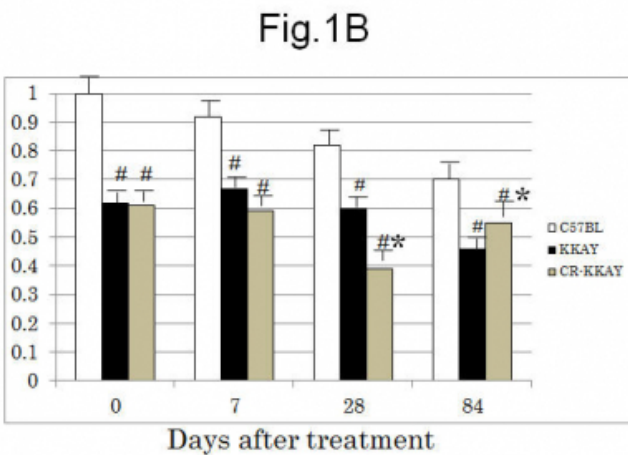
#P<0.05 vs.C57BL wild type mice, \*P<0.05 vs. KKAY obese mice ad libitum.



**Figure 1B**

Cardiac expression of TrkB (Fig.1B) by caloric restriction on KKAY obese mice.

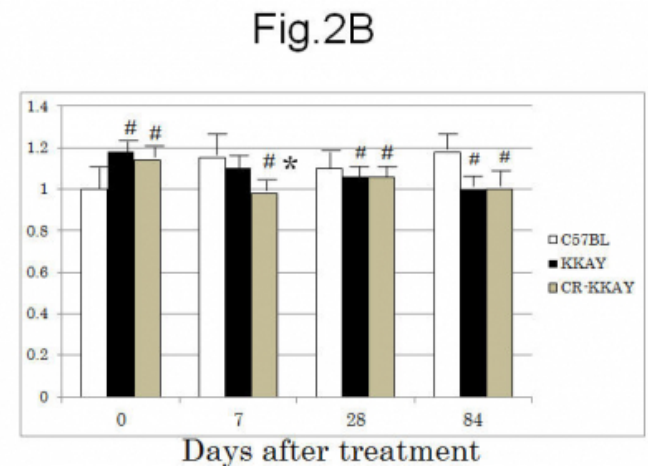
#P<0.05 vs.C57BL wild type mice, \*P<0.05 vs. KKAY obese mice ad libitum.



**Figure 2B**

Hippocampal expression of TrkB (Fig.2B) by caloric restriction on KKAY obese mice.

#P<0.05 vs.C57BL wild type mice, \*P<0.05 vs. KKAY obese mice ad libitum.



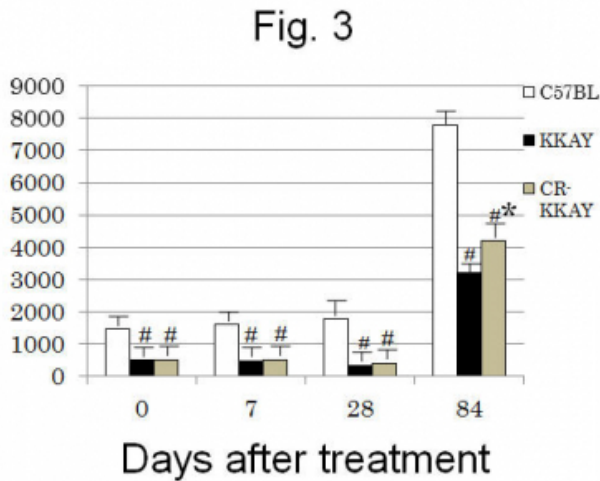
Hippocampal expression of BDNF in CR-KKAY was significantly increased compared with KKAY on day 84. Hippocampal / body weight ratio was also significantly higher in CR-KKAY compared with KKAY on days 28 and 84 (Fig. 2).

The 24-hour spontaneous movement in CR-KKAY was significantly increased compared with KKAY on day 84. Movement in both groups was significantly lower than in C57BL on day 0, 7, 28, and 84 after treatment (Fig. 3).

**Figure 3A**

The 24 hour spontaneous movement by caloric restriction on KKAY obese mice.

#P<0.05 vs.C57BL wild type mice, \*P<0.05 vs. KKAY obese mice ad libitum. The eighty four days treatment with caloric restriction significantly induced voluntary movement on obese mice.



**DISCUSSION**

CR can induce cardiac expression of BDNF with increase of daily activity in obese mice. Cardiac BDNF/TrkB signaling plays a role in recovery from obesity. BDNF/TrkB signaling can improve cardiac dysfunction in obese mice with the induced expression of hippocampal BDNF.

BDNF regulates food intake and body weight; however, it is not useful as a therapeutic because of its short half-life. Chronic activation of its receptor, TrkB, represents an alternative strategy for lowering body weight. Elevated blood pressure was a direct effect of high-dose TrkB antibody treatment rather than secondary to substantial weight loss. High-dose TrkB antibody lowered body weight, whereas low-dose TrkB antibody treatment caused therapeutic weight loss without adverse cardiovascular effects. Dose-dependent TrkB activation lowers body weight and transiently raises blood pressure in mice with diet-induced obesity [9].

Hypothalamic neuron BDNF plays a critical role in energy homeostasis [10]. BDNF, present in the hippocampus, cortex, basal forebrain, many nuclei in the brainstem, and catecholaminergic neurons, including dopamine neurons in the substantia nigra, regulates food intake and body weight in experimental animals and humans.

The therapeutic efficacy of BDNF by gene transfer was demonstrated in mouse models of obesity, which revealed marked weight loss and alleviation of obesity-associated

disorders [11]. These findings indicate that BDNF activates the sympathetic nervous system by the central nervous system and regulates energy expenditure in obese diabetic animals. BDNF has a critical role in obesity and type-2 diabetes mellitus [12].

The increased production of BDNF in the brain cells of rodents on CR likely results from a cellular stress response because BDNF expression is known to be increased by several types of mild stress [13]. CR may enhance signaling pathways in the brain involving BDNF. The BDNF may exert beneficial effects on peripheral glucose regulation and cardiovascular risk factors. In addition, CR may exert more direct beneficial effects on the cells of the blood vessels and the heart.

CR is to date the most effective intervention for improving health, maintaining function and increasing mean and maximum lifespan in a variety of species [14]. Experimental rodents subjected to lifelong CR display up to 60% maximum lifespan extension compared to ad libitum fed controls [14]. CR has been shown to delay the onset of age-related cardiac alterations and ameliorate virtually all known cardiovascular disease (CVD) risk factors both in experimental animals and humans [14,15]. CR may have additional beneficial effects on several metabolic and molecular factors that modulate the cardiovascular system by improving several cardiometabolic risk factors. In the heart, CR protects against fibrosis, reduces cardiomyocyte apoptosis, prevents myosin isoform shifts and preserves or improves left ventricular diastolic function. The benefits of CR, such as protection against obesity, diabetes, hypertension, and cancer, suggest that CR has a major beneficial effect on health span, life span, and quality of life in humans [16,17].

The beneficial role of BDNF in the brain-heart axis has been reported. Activation of neuronal BDNF may be a therapeutic strategy for cardiac disease [18]. BDNF knockout mice showed marked severity of myocardial infarction and deletion of TrkB also exacerbated the cardiac dysfunction. The plasma level of BDNF was associated with brain BDNF, not cardiac BDNF. This report showed that the cardio-protective effects of BDNF are associated with a brain-mediated pathway. In contrast, our data showed that both cardiac and brain BDNF were associated with beneficial effects in physical activity. The BDNF/Trkb signaling in both heart and brain would be associated with cardiac function.

All-cause mortality is higher in obese people, primarily due to increased CVD mortality and increased obesity related

cancer mortality [19]. Rapid increase in the prevalence of obesity is not due to genetic changes but rather to a societal mismatch between physiology and environment, where food is abundant and exercise is unnecessary.

## CONCLUSIONS

In conclusion, our results indicate that food restriction could alter morphological changes in the heart-brain pathway through the increased expression of BDNF/TrkB signaling. Moreover, physical activity is also promoted by chronic food restriction. In humans, the physician can suggest food restriction as a definite strategy for the treatment of heart failure with obesity.

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