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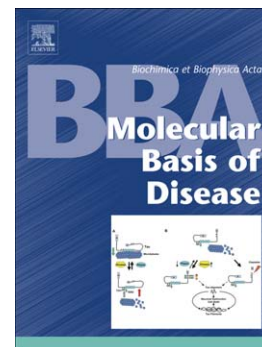
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**Failure of the feeding response to fasting in carnitine-deficient juvenile visceral steatosis (JVS) mice: Involvement of defective acyl-ghrelin secretion and enhanced corticotropin-releasing factor signaling in the hypothalamus**

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*Abbreviations:* aCSF, artificial cerebrospinal fluid; ACTH, adrenocorticotrophic hormone; AGRP, agouti-related protein; CART, cocaine- and amphetamine-regulated transcript; CRF, corticotropin-releasing factor; FAO, fatty acid oxidation; icv, intracerebroventricular; ip, intraperitoneal; JVS, juvenile visceral steatosis; NPY, neuropeptide Y; OCTN2, organic cation transporter 2; POMC, proopiomelanocortin

*Key Words:* anti-famine homeostatic mechanism, fatty acid metabolism abnormalities, ghrelin, hypothalamus-pituitary-adrenal axis

## **ABSTRACT**

Carnitine-deficient juvenile visceral steatosis (JVS) mice, suffering from fatty acid metabolism

abnormalities, have reduced locomotor activity after fasting. We examined whether JVS mice exhibit specific defect in the feeding response to fasting, a key process of anti-famine homeostatic mechanism. Carnitine-deficient JVS mice showed grossly defective feeding response to 24h-fasting, with almost no food intake in the first 4 h, in marked contrast to control animals. JVS mice also showed defective acyl-ghrelin response to fasting, less suppressed leptin, and seemingly normal corticotropin-releasing factor (CRF) expression in the hypothalamus despite markedly increased plasma corticosterone. The anorectic response was ameliorated by intraperitoneal administration of carnitine or acyl-ghrelin, with decreased CRF expression. Intracerebroventricular treatment of CRF type 2 receptor antagonist, antisauvagine-30, recovered the defective feeding response of 24h-fasted JVS mice. The defective feeding response to fasting in carnitine-deficient JVS mice is due to the defective acyl-ghrelin and enhanced CRF signaling in the hypothalamus through fatty acid metabolism abnormalities. In this animal model, carnitine normalizes the feeding response through an inhibition of CRF.

## **1. Introduction**

Juvenile visceral steatosis (JVS) mice, an animal model of systemic carnitine deficiency with genetically defected organic cation transporter 2 (OCTN2), suffer from fatty liver, hypoglycemia, hyperammonemia, growth retardation, and cardiac hypertrophy [1-6]. The abnormalities could be caused by accumulation of long-chain fatty acids, which are not well metabolized due to the disturbed entry into mitochondria in carnitine-deficient state [1-4,7]. As a proof, the abnormalities are ameliorated by intraperitoneal administration of carnitine. Fatty liver and locomotor activity reduction observed in this animal model are deteriorated under fasting condition [8-10] since fatty acids are a major energy source in fasting [10]. Fasting is avoided in the treatment of patients with fatty acid oxidation (FAO) disorders [11,12] in order to prevent accelerated fatty acid metabolism and hypoglycemia.

We have previously reported reduced locomotor activity after fasting in JVS mice, with an involvement of orexin neuronal activity in the hypothalamus [9]. Orexin/hypocretin neuropeptides are produced by a small set of neurons in the lateral hypothalamic and perifornical areas, which are involved in the control of feeding behavior, wakefulness, motor activity, and metabolic and autonomic functions. In fact, orexin was considered to be responsible for the anorexia of JVS mice in the weaning period [13] although the hypothesis needs to be further examined.

A compelling evidence supports the existence of a homeostatic system that dynamically

adjusts energy intake and expenditure to keep body fat mass within a very narrow range [14,15].

The feeding response to fasting and starvation is essential for the survival of all animals and humans and it is thought that energy homeostasis operates primarily to defend against weight loss rather than weight gain over the course of evolution. Through the detailed analysis on feeding behavior of JVS mice, we found that the mice do not show anorexia under free feeding condition different from during weaning period [13], but fail to respond to 24h-fasting in marked contrast to normal animals. We now report that the defective secretion of acyl-ghrelin, an appetite-stimulatory gastric hormone [16,17], and increased activity of anorexigenic corticotrophin-releasing factor (CRF) in the hypothalamus of the animals underlie the defective feeding response to fasting and failure of the anti-famine homeostatic mechanism.

## **2. Materials and methods**

### 2.1. Animals

Wild and homozygous mutant JVS mice were obtained by cross-mating the heterozygous mice or by mating homozygous mutant male and heterozygous female mice. The mice residing in a closed colony originated from C3H.OH strain were used for all experiments. The mice were kept at  $22 \pm 1.0$  °C with free access to water and food (CE-2; CLEA Japan, Tokyo, Japan) and with lights on 12 h/day (0700-1900 h). Neonatal (7-28 days of age) JVS mice were treated intraperitoneally with L-carnitine-HCl (5  $\mu$ mol/mouse; Sigma-Aldrich, St. Louis, MO), dissolved in physiological saline and neutralized with 0.2 M NaOH, as described previously [2]. For the homozygous mutant mice, 2-3 mice were kept in one cage to suppress the mortality. At 8 weeks of age, the mutant mice were housed individually. Male mice at 10-16 weeks of age were used for all experiments.

This study was approved by the Ethical Committee for Animal Experimentation at Kagoshima University, which was standardized to Japanese national guidelines for animal experiments.

### 2.2. Genotyping and operation for intracerebroventricular injection

For genotyping, genomic DNA was extracted with a buffer [50 mM NaCl, 10 mM Tris-

HCl, 2.5 mM MgCl<sub>2</sub>, 0.45% (v/v) NP40, 0.45% (v/v) Tween 20, pH 8.4] from part of their ear.

The L352R mutation of *Slc22a5* (GenBank: AF111425) in the mutant mice was detected by amplification with primers; 5'-AGATGCACTGGCAAAGGTTATA-3' (intron 5) and 5'-TTCAACAGCCGCCAGTAGGA-3' (exon 6), followed by digestion with *Cfr*13I (Bio-Rad, Hercules, CA) [9].

For intracerebroventricular (icv) administration, the anesthetized mice were placed in a David Kopf stereotaxic apparatus with a mouse adapter. The stereotaxic coordinates for lateral cerebroventricular injections were 0.9 mm posterior to bregma, 0.9 mm lateral to midline, and 4.2 mm below the dura [18,19]. The mice were allowed to recover for at least 1 week after this operation.

### 2.3. Preparation of drugs

For intraperitoneal (ip) administration, L-carnitine·HCl (10 μmol/mouse) was dissolved in physiological saline and neutralized with 0.2 M NaOH. Acyl-ghrelin (10 nmol/mouse, Yanaihara Institute Inc., Shizuoka, Japan) was dissolved in physiological saline. For icv administration, neuropeptide Y (NPY: 3.0 μg/mouse, Yanaihara Institute Inc., Shizuoka, Japan) and anti-sauvagine-30 (CRF type 2 receptor antagonist, 0.75 μg/mouse; Polypeptide

Laboratories, Torrance, CA) were dissolved in artificial cerebrospinal fluid (aCSF: 138.9 mM NaCl, 3.4 mM KCl, 1.26 mM CaCl<sub>2</sub>, 4.0 mM NaHCO<sub>3</sub>, 0.6 mM NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 5.6 mM Glucose) [18,19]. Carnitine (100 nmol/mouse; Sigma-Aldrich) was dissolved in physiological saline.

#### 2.4. Food intake

The cumulative food intake was examined in mice under free-feeding and re-feeding after 24h-fasting. Measurements were started at 0700 h except as otherwise indicated. Before re-feeding, food was deprived from mice for 24 h, with free accessing to water. After the ip administration of vehicle (150 µl of saline), acyl-ghrelin (10 nmol/150 µl of saline) or carnitine (L-carnitine-HCl neutralized with NaOH, 10 µmol/150 µl) at 0700 h, or the icv administration of 4 µl of vehicle (saline or aCSF) or indicated chemicals at 0700 h, the cumulative food intake was calculated for indicated hour. Food intake was determined by calculating the difference between the weight of supplied standard chow and the weight of remained chow and visible split at the end of each time point.

#### 2.5. Measurement of metabolic parameters and hormone concentrations

The body weight was measured at 0700-0900 h. For measurement of metabolic parameters and hormone concentration except ghrelin, ACTH and corticosterone, the mice were anesthetized with sodium pentobarbital (100 mg/kg, ip) and blood samples were collected from heart using syringe containing EDTA (5  $\mu$ l of 200 mM) between 0700 and 0900 h. For ghrelin, ACTH and corticosterone, blood drops were collected into tubes containing EDTA (5  $\mu$ l of 200 mM) after decapitation. Plasma was stored at -80 °C until analysis. After the mice were killed, the hypothalamic block, stomach, and epididymal fat were removed, and then frozen in liquid nitrogen. They were stored at -80 °C until RNA extraction. Plasma glucose and free fatty acids (FFA) were measured by kits using glucose oxidase and acyl-CoA oxidase, respectively (Wako, Osaka, Japan). Plasma insulin (Morinaga, Kanazawa, Japan), leptin (R & D system, Minneapolis, MN), acyl-ghrelin (Mitsubishi Kagaku, Tokyo, Japan), desacyl-ghrelin (Mitsubishi Kagaku), corticosterone (Assay Designs, Ann Arbor, MI) and ACTH (MD biosciences, St. Paul, MN) were measured by the respective kit based on enzyme immunoassay.

#### *2.6. Real-time PCR for quantification of mRNA*

Mice were fed or fasted for 24 h. The mice were killed by decapitation or cervical dislocation. Total RNA was isolated from the hypothalamic block, stomach and epididymal fat

with the method of Chomczynski and Sacchi (20). Isolated RNA was treated with DNAase treatment to delete genomic contamination. First-strand cDNA synthesis was performed using 5 µg of total RNA and oligo-(dT)<sub>12-18</sub> priming following the manufacture's instructions (Invitrogen, Carlsbad, CA). Real-time quantitative PCR was performed using SYBR-green chemistry on a TAKARA detection system (TAKARA, Shiga, Japan). Reactions were performed under the standard conditions recommended by the manufacture. We used the mouse cyclophilin gene as an internal control for all samples. All expression data were normalized to the level of cyclophilin expression from the individual sample. The following primers were used for real-time PCR: Cyclophilin forward 5'-GGTGGAGAGCACCAAGACAGA-3', reverse 5'-GCCGGAGTCGACAATGATG-3' [21]; Leptin forward 5'-CTGTGGCTTTGGTCCTATCT-3', reverse 5'-TGATAGACTGCCAGAGTCTG-3' [22]; Ghrelin forward 5'-AGGAGCTGGAGATCAGGTTCAAT-3', reverse 5'-CTGCTGATACTGAGCTCCTGACAG-3' [23]; NPY forward 5'-CTCCGCTCTGCGACACTAC-3', reverse 5'-AATCAGTGTCTCAGGGCT-3' [21]; AGRP forward 5'-GCGGAGGTGCTAGATCCA-3', reverse 5'-AGGACTCGTGCAGCCTTA-3' [21]; POMC forward 5'-GGCTTGCAAACCTCGACCTCT-3', reverse 5'-TGACCCATGACGTA CT TCCG-3' [24]; CART forward 5'-GCAGATCGAAGCGTTGCAA-3', reverse 5'-TTGGCCGTA CT TCTTCTCGTAGA-3' [24]; Orexin A forward 5'-CGTAACTACCACCGCTTTAGCA-3', reverse 5'-

TGCCATTACCAAGAGACTGACAG-3' [24]; CRF forward 5'-  
CCGGGCAGAGCAGTTAGC-3', and reverse 5'-CAACATTTCATTCCCGATAATCTC-3'  
[25].

### 2.7. Statistical analysis

Results are expressed as the mean  $\pm$  standard deviation (SD). □ The significance of differences between the groups was evaluated by un-paired Student *t* test or one-way analysis of variance (ANOVA) followed by Tukey-Kramer method for multiple comparison. JMP software (SAS Institute, Tokyo, Japan) was used for statistical analysis.

## 3. Results

### 3.1. Food intake of wild type and JVS mice under free-feeding or re-feeding condition after 24h-fasting

Both wild type and JVS mice showed an equivalent food intake in a day under free-feeding

condition (Fig. 1A). However, JVS mice significantly took more food in light phase and less in dark phase than wild type mice. After 24h-fasting, wild type mice took more foods in the first 1, 2, 4, 12, and 24 h, as compared with food intake under free-feeding condition (Fig. 1B). On the other hand, JVS mice failed to show the feeding response to 24h-fasting during 4h-observation period and showed significantly decreased feeding response at 12 and 24 h.

### *3.2. Metabolic and hormone analysis in wild type and JVS mice*

Both wild type and JVS mice had no difference in body weight and epididymal fat weight under free-feeding condition (Table 1). JVS mice had significantly higher FFA and desacyl-ghrelin and lower insulin in the blood than in control animals. After 24h-fasting, they lost body weight and epididymal fat weight similarly, as reflected in lower glucose and insulin and higher FFA and corticosterone levels (Table 1). However, JVS mice had markedly increased FFA, leptin, and corticosterone after fasting than in control animals. The plasma glucose levels did not differ significantly under fasted and fed conditions in JVS and control mice. It was surprising in JVS mice that orexigenic acyl-ghrelin did not respond to fasting and most of the ghrelin forms in the blood is anorexigenic desacyl-ghrelin [19,24], as shown in the markedly increased ratio of desacyl-ghrelin to acyl-ghrelin compared to controls (Table 1).

### *3.3. Effects of carnitine, acyl-ghrelin, and NPY administration on food intake in 24h-fasted wild type and JVS mice*

Ip administration of carnitine (10  $\mu$ mol) or acyl-ghrelin (10 nmol) normalized the decreased feeding response to 24h-fasting in JVS mice, at the doses that did not affect the food intake in wild type animals (Fig. 2A). In contrast, icv administration of carnitine (100 nmol) had no effect in 24h-fasted JVS mice, indicating that carnitine acts in the periphery (Fig. 2B). Icv administration of carnitine (1  $\mu$ mol) to 24h-fasted JVS mice showed no significant effects (data not shown). On the other hand, icv administration of orexigenic NPY that is situated downstream from ghrelin [26], markedly increased food intake in JVS mice under the re-feeding condition (Fig. 2B).

### *3.4. mRNA levels of leptin in white adipose tissue (WAT), ghrelin in stomach, and appetite-regulated peptides in hypothalamus*

Leptin expression in the WAT decreased and ghrelin expression in the stomach increased after fasting in wild type animals (Fig. 3 A, B), in accordance with plasma concentrations of the

respective hormones (Table 1). However, in JVS mice, leptin expression paradoxically increased after fasting, with no change of ghrelin, which is quite different from controls. JVS mice also showed increased expression of orexigenic NPY/AGRP and decreased anorexigenic POMC in the hypothalamus (Fig. 3 C-H), suggesting the existence of some adaptive response to fasting in this animal model. Neither CART nor orexin expression is altered after fasting both in wild type and JVS mice. However, despite markedly increased corticosterone levels, CRF expression of JVS mice remained at similar levels to controls in fasting and free-feeding conditions, suggesting the increased CRF activity in this animal model.

### *3.5. Effects of carnitine or acyl-ghrelin administration on feeding-regulated peptides in 24h-fasted JVS mice*

To get further insight into the defective anti-famine homeostatic mechanisms in JVS mice, we examined changes of feeding-regulatory peptides after ip administration of carnitine and acyl-ghrelin. Carnitine failed to increase levels of acyl-ghrelin in the blood and NPY in the hypothalamus and to decrease levels of leptin in the blood and POMC in the hypothalamus (Fig. 4 A-F). Acyl-ghrelin administration also failed to increase NPY and to decrease leptin and POMC expression: however, both carnitine and acyl-ghrelin significantly decreased CRF

expression in the hypothalamus of JVS mice (Fig. 4G).

### *3.6. Effects of CRF receptor antagonist on food intake in 24h-fasted JVS mice*

To examine whether increased CRF signaling is involved in the defective feeding response to fasting, we examined effects of CRF type 2 receptor antagonist on food intake in 24h-fasted JVS mice (Fig. 5). There are two types of CRF receptors in the central nervous system in mammals [27,28], but the type 2 receptor is the chief receptor mediating the anorexic effects of CRF [29, 30]. Icv anti-sauvagine-30 significantly increased food intake in JVS mice at the dose that did not affect food intake in wild type animals (Fig. 5).

## **4. Discussion**

We found that carnitine-deficient JVS mice exhibited defective feeding response to fasting, a key process known as anti-famine homeostatic mechanisms (Fig. 6). Administration of acyl-ghrelin and carnitine normalized the feeding response of 24h-fasted JVS mice, with an inhibition of CRF expression in the hypothalamus. Furthermore, CRF type 2 receptor antagonist greatly improved the feeding response in fasted JVS mice, indicating that the defective acyl-

ghrelin secretion and increased CRF signaling would underlie the feeding abnormalities of the animals. Ghrelin is a gastric hormone linked to meal initiation, energy expenditure, and fuel partitioning, and is considered to facilitate weight gain and impede weight loss as an indicator of energy insufficiency [31,32]. The lowered acyl-ghrelin with heightened desacyl form suggests the decrease in acylation and/or the increase in desacylation for processing of preproghrelin. The acylation process normally requires medium-chain fatty acids [33] and will be interfered with markedly increased long-chain fatty acids that are difficult to be metabolized in JVS mice and FAO disorder patients. The disturbed synthesis and secretion of leptin might also be produced by similar mechanisms, although leptin may have a limited role in the defective feeding response since carnitine- and ghrelin-induced food intakes appear not to involve leptin.

The expression of orexigenic and anorexigenic peptides in the hypothalamus of fasting JVS mice showed some adaptive response to fasting, i.e., increased NPY/AGRP and decreased POMC [34,35], which may be due to defective substrate availability such as glucose and fatty acids. These findings are consistent with the data by Kuwajima et al. observed in the infantile period of JVS mice [13] although they did not discriminate fasted and fed conditions. Recently, Obici et al. indicated that fatty acids metabolism in the hypothalamus plays an important role in the control of feeding behavior [36-38]. In these reports, accumulated fatty acids in the

hypothalamus inhibited food intake through decreased expression of orexigenic NPY and AGRP. However, the interplay between hypothalamic fatty acid signals and feeding behavior appears to be complex and how manipulations of central nervous system fatty acid metabolism are translated into changes in organism energy balance remains to be determined [39]. Our findings of the increased expression of NPY/AGRP in fasted JVS mice suggest that fatty acid oxidation rather than accumulation is important in the hypothalamic energy perception. It is likely that the increased orexigenic activity by NPY/AGRP was canceled by paradoxically increased anorexigenic activity such as CRF and leptin during fasting in this animal model.

Icv administration of carnitine failed to improve anorexia of fasted JVS mice, in marked contrast to the ip administration. Ip administration of carnitine increased brain carnitine concentrations very slightly [10,12]. We have reported the effect of ip administration of carnitine on plasma and tissue carnitine levels [10]. Ten  $\mu$ mol of ip administration to 24h-fasted JVS mice increased the carnitine levels largely in liver and plasma, but slightly in heart skeletal muscle and brain [10]. Therefore, carnitine should act to suppress the enhanced CRF signaling through decrease in FFA in the blood and the hypothalamus [39] leading to initiation of feeding. Actually, FFA in 24h-fasted JVS mice decreased after ip administration of carnitine as previously reported [10]. Accumulated fatty acids in the hypothalamus and peripheral tissues such as liver produce fatty liver, cardiac hypertrophy, and reduced locomotor activity, in

addition to defective feeding response to fasting (Fig. 6). Fasting should be avoided as much as possible in patients with FAO disorders since hypoglycemia may be fatal in some cases. Carnitine-deficient JVS mice may be a good model not only for FAD patients, but also for cahectic cancer and other patients who also show defective feeding response to fasting/starvation with secondary hypocarnitinemia [40, 41].

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**Figure Legends**

**Fig. 1.** (A): Food intake of wild type (open column, n = 16) and JVS (closed column, n = 13) mice under free-feeding condition was measured in light and dark phases (light, 0700-1900 h; dark, 1900-0700 h; total, 0700-0700 h at the next day). Results are expressed as mean  $\pm$  SD. \*,  $p < 0.05$  vs. wild type mice of the respective phases by Student *t* test. (B): Food intake of fed or 24h-fasted wild type (open column, fed: n = 16, fasted: n = 16) and JVS (closed column, fed: n = 13, fasted: n = 14) mice was measured (1 h, 0700-0800 h; 2 h, 0700-0900 h; 4 h, 0700-1100 h; 12 h, 0700-1900 h; 24 h, 0700-0700 h at the next day). Results are expressed as mean  $\pm$  SD. \*,  $p < 0.05$  vs. wild type mice of the same feeding condition by Student *t* test. #,  $p < 0.05$  vs. fed condition of the respective genotypes by Student *t* test.

**Fig. 2.** (A): Effect of intraperitoneal administration of saline (Na, 150  $\mu$ l), carnitine (Cn, 10

$\mu\text{mol}/150\mu\text{l}$ ) or acyl-ghrelin (Ghr, 10 nmol/150 $\mu\text{l}$ ) on cumulative food intake after 24h-fasting in wild type (open column, Na: n = 13, Cn: n = 13, Ghr: n = 4) and JVS (closed column, Na: n = 11, Cn: n = 9, Ghr: n = 8) mice (1 h, 0700-0800 h; 2 h, 0700-0900 h; 4 h, 0700-1100 h; 12 h, 0700-1900 h; 24 h, 0700-0700 h at the next day). Results are expressed as mean  $\pm$  SD. \*,  $p < 0.05$  vs. wild type mice of the same treatment by Student *t* test. The differences between the treatments in the same genotype at each time point were assessed by one-way ANOVA. Values sharing identical superscripts (alphabetical or Greek letter) are not significantly different. (B): Effect of intracerebroventricular administration of aCSF (4  $\mu\text{l}$ , n = 4), carnitine (Cn, 100 nmol/4  $\mu\text{l}$ , n = 5) or NPY (10 nmol/4  $\mu\text{l}$ , n = 4) on cumulative food intake (1 h, 0700-0800 h; 2 h, 0700-0900 h) after 24h-fasting in JVS mice (closed column). #,  $p < 0.05$  vs. aCSF injected mice at each time point.

**Fig. 3.** mRNA levels of leptin in the white adipose tissue (WAT) (A), ghrelin in the stomach (B), NPY (C), AGRP (D) POMC (E) CART (F), orexin (G), and CRF (H) in the hypothalamus of wild type (open column) and JVS (closed column) mice were measured by real-time PCR. The mRNA levels relative to cyclophilin were calculated with the value of fed wild type mice set at 1.0. The numbers of samples are from 4 to 12. Results are expressed as mean  $\pm$  SD. \*,  $p < 0.05$  vs. wild type mice of the same feeding condition by Student *t* test. #,  $p < 0.05$  vs. fed condition of

the respective genotypes by Student *t* test.

**Fig. 4.** □ Effect of intraperitoneal (ip) administration of saline (Na, 150  $\mu$ l) or carnitine (10  $\mu$ mol/150  $\mu$ l) on acyl-ghrelin (A), desacyl-ghrelin (B) and ratio of desacyl-ghrelin to acyl-ghrelin (C) in the blood of 24h-fasting wild type (open column, n = 4) and JVS (closed column, n = 6) mice. Effect of ip administration of saline (Na, 150  $\mu$ l), carnitine (Cn, 10  $\mu$ mol/150  $\mu$ l) or acyl-ghrelin (Ghr, 10 nmol/150  $\mu$ l) on leptin in the blood (D) of 24h-fasted wild type (n = 4) and JVS mice (n = 6). Effect of ip administration of saline (Na, 150  $\mu$ l), carnitine (Cn, 10  $\mu$ mol/150  $\mu$ l) or acyl-ghrelin (Ghr, 10 nmol/150  $\mu$ l) on mRNA levels of NPY (E), POMC (F) and CRF (G) in the hypothalamus of 24h-fasted JVS mice by real-time PCR (n = 6). The mRNA levels relative to cyclophilin were calculated with the value of saline-administered JVS mice set at 1.0. All samples were collected at 2 h after the administration of chemicals to the mice. \*,  $p < 0.05$  vs. wild type mice of the same treatment by Student *t* test. #,  $p < 0.05$  vs. saline administered 24h-fasted JVS mice by Student *t* test.

**Fig. 5.** Effect of intracerebroventricular administration of aCSF (4  $\mu$ l) or CRF type 2 receptor-antagonist (CRF<sub>2</sub>RA, anti-sauvagine-30, 0.75  $\mu$ g/4  $\mu$ l of aCSF) on cumulative food intake (2 h, 0700-0900 h; 4 h, 0700-1100 h) in 24h-fasted wild type (aCSF, n = 5; antagonist, n = 3) and

JVS (aCSF, n = 7; antagonist, n = 7) mice. \*,  $p < 0.05$  vs. wild type mice of the same treatment at each time point by Student  $t$  test. #,  $p < 0.05$  vs. aCSF administered mice of the respective genotypes at each time point by Student  $t$  test.

**Fig. 6.** A simplified model for the defective feeding response to fasting in JVS mice. Fatty acids are predominant substrate for energy production during fasting and starvation. Carnitine is required for transfer of long-chain fatty acids from cytoplasm to mitochondrial matrix for their oxidation. Lack of plasma membrane carnitine transporter in this animal model and humans results in urinary carnitine wasting and defective fatty acids oxidation, thereby leading to fat accumulation/ impaired function in liver, heart and skeletal muscle, as well as decreased ketone and glucose production. Normally, a loss of body fat during fasting decreases leptin, which leads to positive energy balance by increased production, release, and/or action of orexigenic acyl-ghrelin and NPY/AGRP, as well as by decreased activity of anorexigenic POMC and CRF. In JVS mice, the increased fatty acids disrupt this anti-famine homeostatic mechanism through decreased acyl ghrelin and disinhibited leptin/CRF, which outweighs some adaptive orexigenic NPY/AGRP response induced by defective substrate availability (glucose) and beta oxidation (FFA). The net effect is thus complex and potential changes in newly identified feeding-regulatory pathways or energy sensing mechanisms such as hypothalamic adenosine

monophosphate-activated protein kinase (AMPK) activity [37,42] need to be examined.

Carnitine normalized the feeding response to fasting through decreased FFA in the blood and

lowered CRF expression in the hypothalamus.

ACCEPTED MANUSCRIPT

**Table 1** Physiological and biochemical analyses of wild type and JVS mice

	Fed		Fasting	
	Wild	JVS	Wild	JVS
Body weight (g)	24.7 ± 2.6	24.9 ± 2.6	21.3 ± 2.4 <sup>#</sup>	20.6 ± 1.7 <sup>#</sup>
Epididymal fat (mg)	199 ± 99	252 ± 96	109 ± 56 <sup>#</sup>	120 ± 55 <sup>#</sup>
Glucose (mg/dl)	161 ± 16	165 ± 9	130 ± 18 <sup>#</sup>	118 ± 40 <sup>#</sup>
FFA (μEq/l)	110 ± 40	260 ± 110*	345 ± 111 <sup>#</sup>	2487 ± 1380 <sup>*,#</sup>
Insulin (ng/ml)	0.57 ± 0.33	0.18 ± 0.98*	0.14 ± 0.17 <sup>#</sup>	0.05 ± 0.04 <sup>#</sup>
Leptin (ng/ml)	3.35 ± 0.77	6.26 ± 3.60	0.32 ± 0.19 <sup>#</sup>	2.67 ± 2.26*
Acyl-Ghrelin (fmol/ml)	38 ± 11	40 ± 13	379 ± 309 <sup>#</sup>	25 ± 15*
Desacyl-Ghrelin (fmol/ml)	271 ± 35	1013 ± 292*	1468 ± 427 <sup>#</sup>	1635 ± 984
Desacyl- to Acyl-Ghrelin	7.7 ± 2.1	22.2 ± 4.1*	5.2 ± 2.4	73.2 ± 26.1 <sup>*,#</sup>
Corticosterone (ng/ml)	1.2 ± 0.3	109 ± 130	140 ± 104 <sup>#</sup>	517 ± 376 <sup>*,#</sup>
ACTH (pg/ml)	16.7 ± 7.8	27.5 ± 16.2	26.1 ± 16.0	2.8 ± 5.5 <sup>*,#</sup>

Results are expressed as mean ± SD (n = 6). Fed and fasting mean free-feeding and 24h-food deprivation condition, respectively. \*,  $p < 0.05$  vs. wild type mice of the same feeding condition by Student *t* test. #,  $p < 0.05$  vs. fed condition of the respective genotypes by Student *t* test.

Fig. 1.

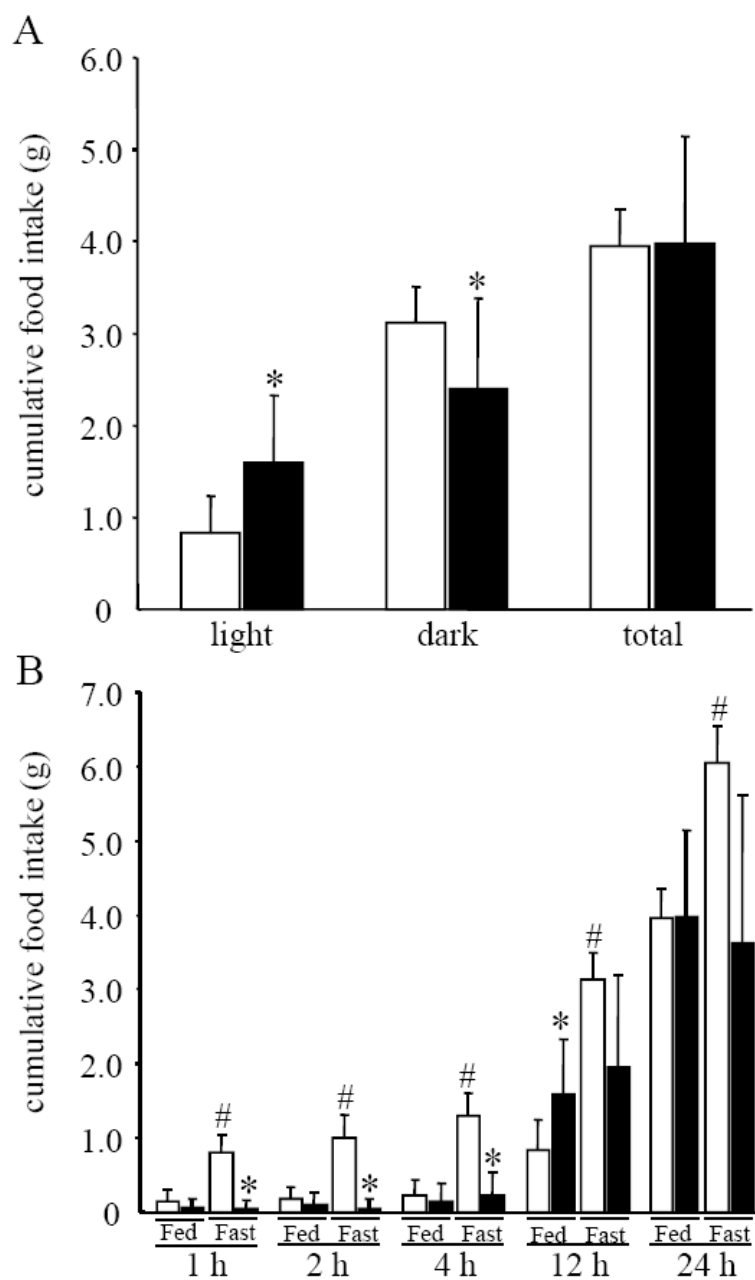




Fig. 3.

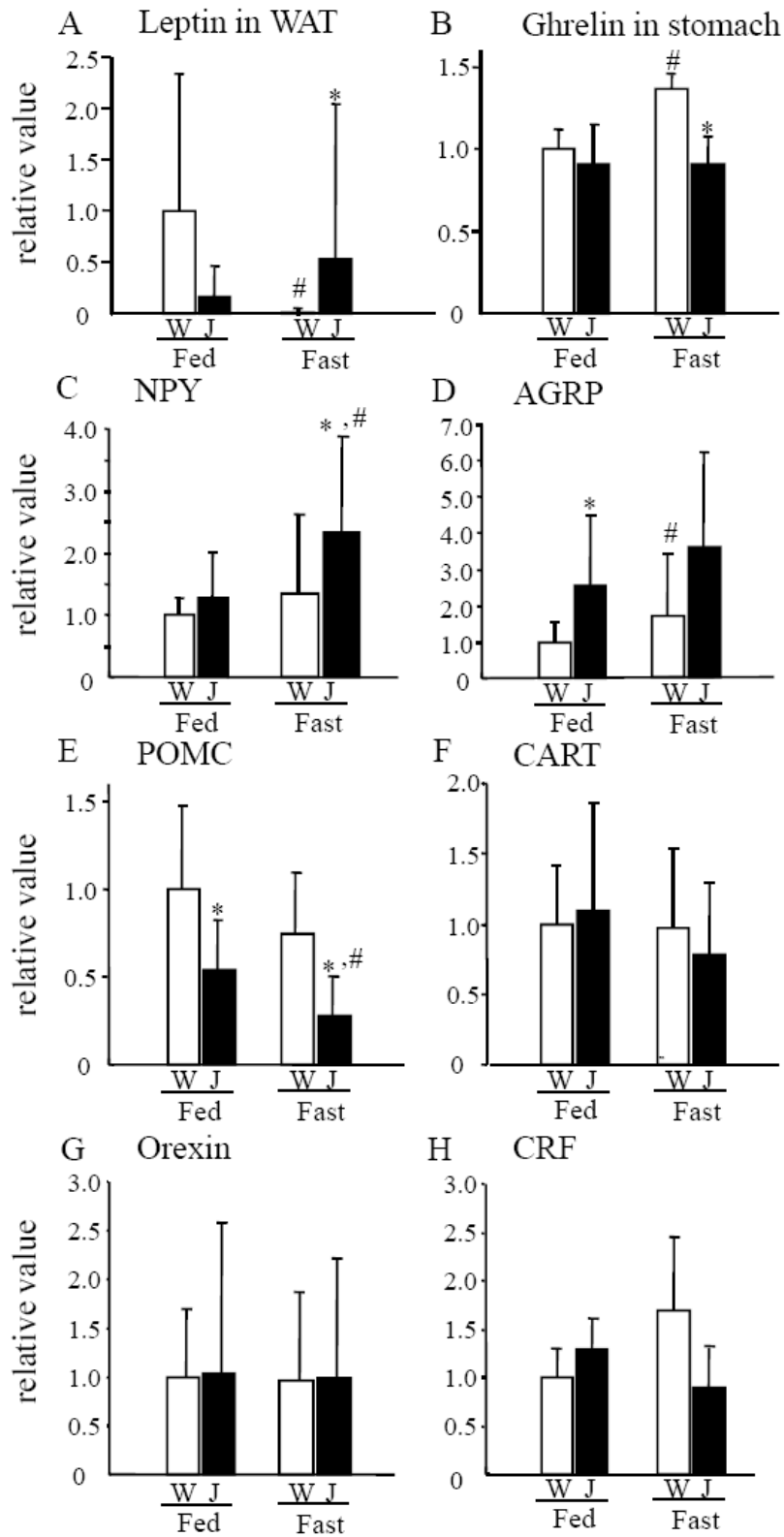


Fig. 4.

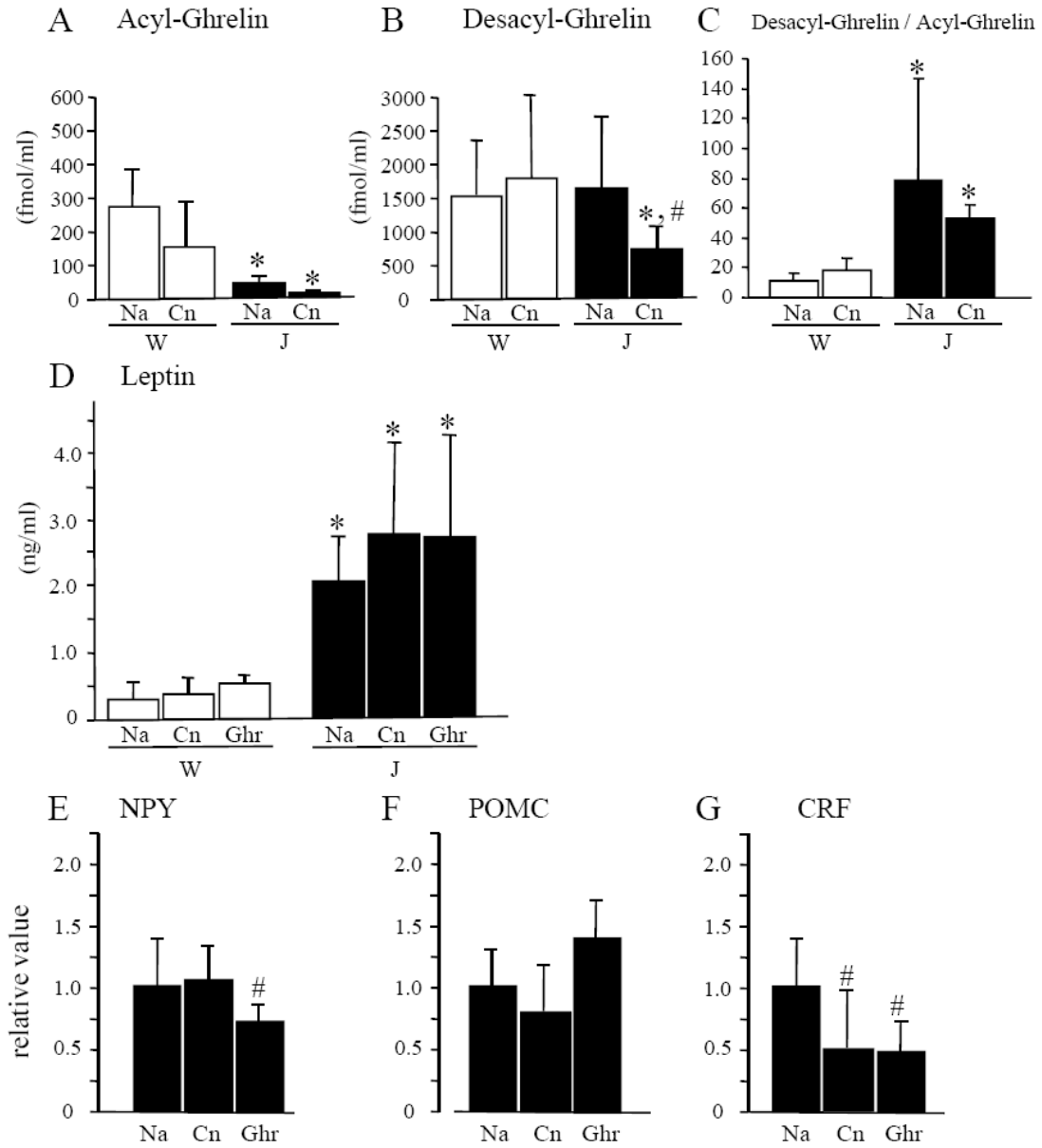


Fig. 5.

