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Brief Report

Primary cilia regulate adaptive responses to fasting

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ABSTRACT

Objective: Neuronal primary cilia are known to be a required organelle for energy balance and leptin action. However, whether primary cilia directly mediate adaptive responses during starvation is yet unknown. Therefore, we investigated the counterregulatory roles of primary cilia, and their related leptin action in energy-depleted condition.

Method: We generated leptin receptor (LepR) neuron-specific primary cilia knockout (*Ift88* KO^{LepR}) mice. Leptin-mediated electrophysiological properties of the neurons in fasting condition were assessed using patch-clamp technique. Adaptive responses and neuroendocrine reflexes were measured by monitoring counterregulatory hormones.

Results: In fasting state, the leptin-induced neuronal excitability and leptin homeostasis were impaired in *Ift88* KO^{LepR}. In addition, the *Ift88* KO^{LepR} exhibited aberrant fasting responses including lesser body weight loss, decreased energy expenditure, and lower heat generation compared to wild-type littermates. Furthermore, the primary cilia in LepR neurons are necessary for counterregulatory responses and leptin-mediated neuroendocrine adaptation to starvation.

Conclusion: Our results demonstrated that the neuronal primary cilia are crucial neuronal components mediating the adaptive counterregulatory responses to starvation.

1. Introduction

Starvation is a potential threat for living organisms. Thus, animals including humans have developed an efficient homeostatic defense mechanism to cope with decreasing energy stores [1]. During the initial phase of fasting, the counterregulatory hormones including glucagon, catecholamines, and corticosterone are released to maintain body homeostasis [2,3]. Along with those hormones, a decrease of leptin level during fasting is a critical signal to activate normal counterregulatory neuroendocrine reflexes [2,4].

Primary cilia have been identified as crucial organelles for regulating metabolic homeostasis [5–7]. Primary cilia are also functional components for normal leptin action [8,9]. However, it is unknown whether the neuronal primary cilia play any homeostatic role in fasting condition, or whether the cilia have any roles in sensing metabolic status in an energy-depleted condition.

In this study, we demonstrated that primary cilia in leptin receptor neurons are required for proper metabolic transitions to a starved condition. We uncovered the primary cilia in neurons are necessary not only for counterregulatory responses to a fasting condition but also for the leptin-mediated neuroendocrine reflexes.

2. Methods

2.1. Mouse models

To specifically delete the intraflagellar transport 88 (*Ift88*) gene from leptin receptor (LepR)-expressing neurons, we crossed transgenic LepR-

Abbreviations: EE, energy expenditure; Ift88, intraflagellar transport 88; KO, knockout; LepR, leptin receptor; RER, respiratory exchange ratio; VO_2, oxygen consumption; WT, wild-type.

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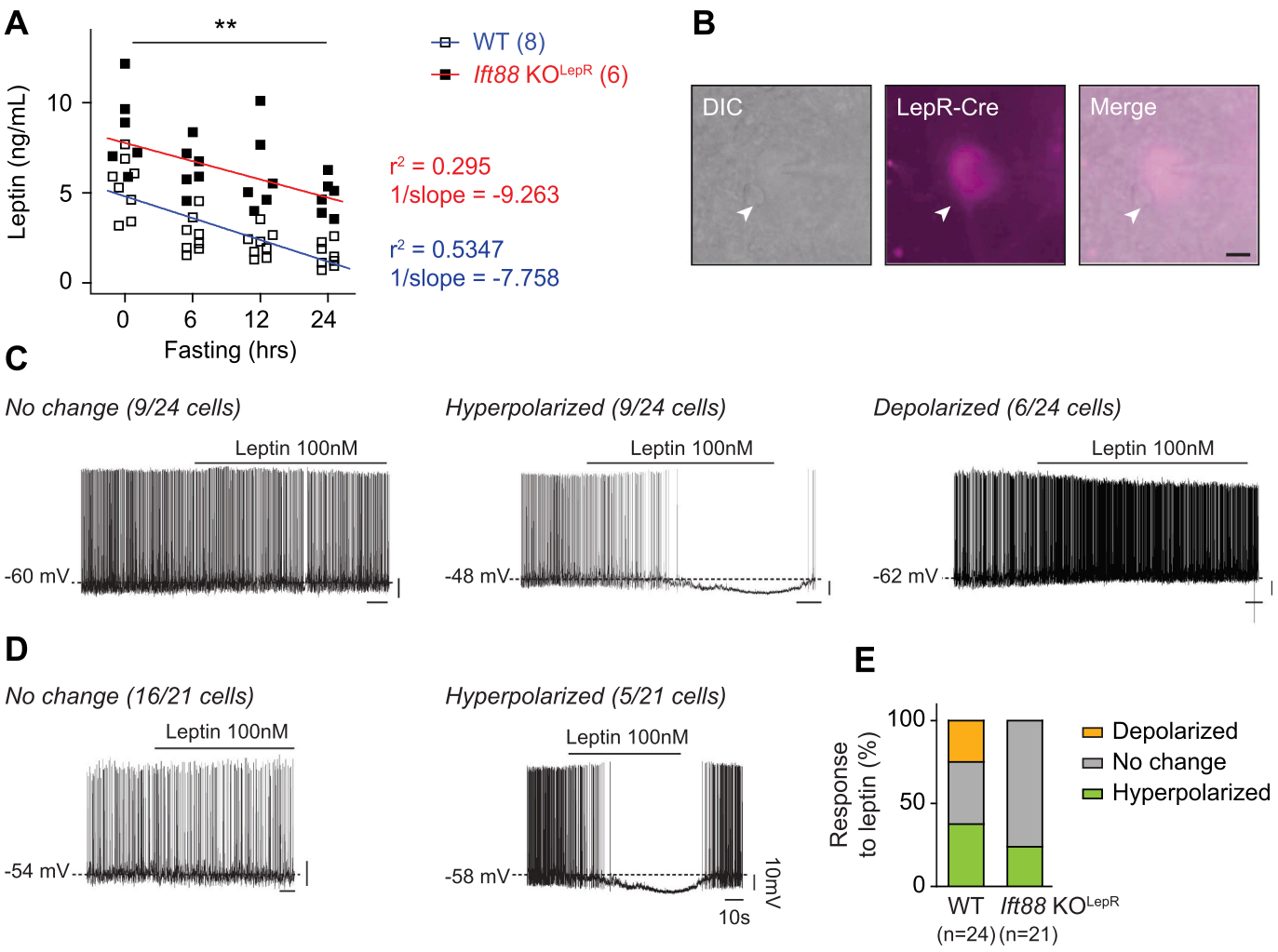

Fig. 1. Primary cilia expressed in LepR neurons are necessary for leptin homeostasis in fasting condition.
(A) Correlation analysis of serum leptin levels during the period of fasting. **p = 0.0018, 2-way ANOVA.
(B) Differential interference contrast (DIC), fluorescence, and merged images of targeted LepR neurons. Arrows indicate the recorded cell. Scale bar: 10 μm.
(C) Representative electrophysiological traces demonstrating no change, hyperpolarization, and depolarization in responses to leptin in the hypothalamic LepR neurons of WT mice.
(D) Representative electrophysiological traces exhibiting no change and hyperpolarization in responses to leptin in the hypothalamic LepR neurons of the *Ift88* KO^{LepR} mice.
(E) Percent in electrophysiological responses in WT and *Ift88* KO^{LepR} mice.
The number of cell and mice used was expressed in parenthesis. The results are expressed as mean ± SD.

Cre heterozygous mice (B6.129(Cg)-*Lepr*$^{tm2(cre)Rck}$/J, Stock No. 008320, Jackson Laboratory, ME, USA) with homozygous for the floxed Ift88 allele mice (B6.129P2-*Ift88*^{tm1Bky}/J, Stock No. 022409, Jackson Laboratory). To visualize the LepR-expressing cell, we bred the LepR-Cre mice with the Cre-inducible tdTomato-reporter mice (B6;129S6-*Gt (ROSA)26Sor*$^{tm9(CAG-tdTomato)Hze}$/J, Stock No. 007905, Jackson Laboratory).

Additional details regarding methods can be found in Supplementary information.

3. Results

3.1. The *Ift88* KO^{LepR} exhibited impaired leptin homeostasis in fasting condition

As neuronal cilia are known to be essential organelles in maintaining body weight and leptin homeostasis [6,9–11], we hypothesized that the primary cilia in the leptin-responsive neurons might play important roles in regulating whole body energy homeostasis. Targeted disruption of *Ift88* gene in leptin receptor (LepR) neurons (*Ift88* KO^{LepR}) induced specific ciliary deletion only in the LepR-expressing neurons (Supplementary Fig. 1A and B). The *Ift88* KO^{LepR} displayed no difference in energy homeostasis including body temperature, food intake, oxygen consumption (VO_2), energy expenditure (EE), respiratory exchange ratio (RER), and locomotion compared to the wild-type (WT) littermates implying that the primary cilia expressed in LepR neurons might have no prominent effects on the body homeostasis in normally fed state (Supplementary Fig. 1C–I). Interestingly, however, the leptin levels of the *Ift88* KO^{LepR} were significantly increased during the whole period of fasting (Fig. 1A). In addition, the blunted activation of p-STAT3 in the *Ift88* KO^{LepR} suggested leptin resistance in the hypothalamus (Supplementary Fig. 2A).

3.2. Primary cilia are required for leptin-mediated neuronal excitability in fasting condition

To further characterize the impaired leptin homeostasis in *Ift88* KO^{LepR}, leptin-mediated neuronal excitability was examined in fasting

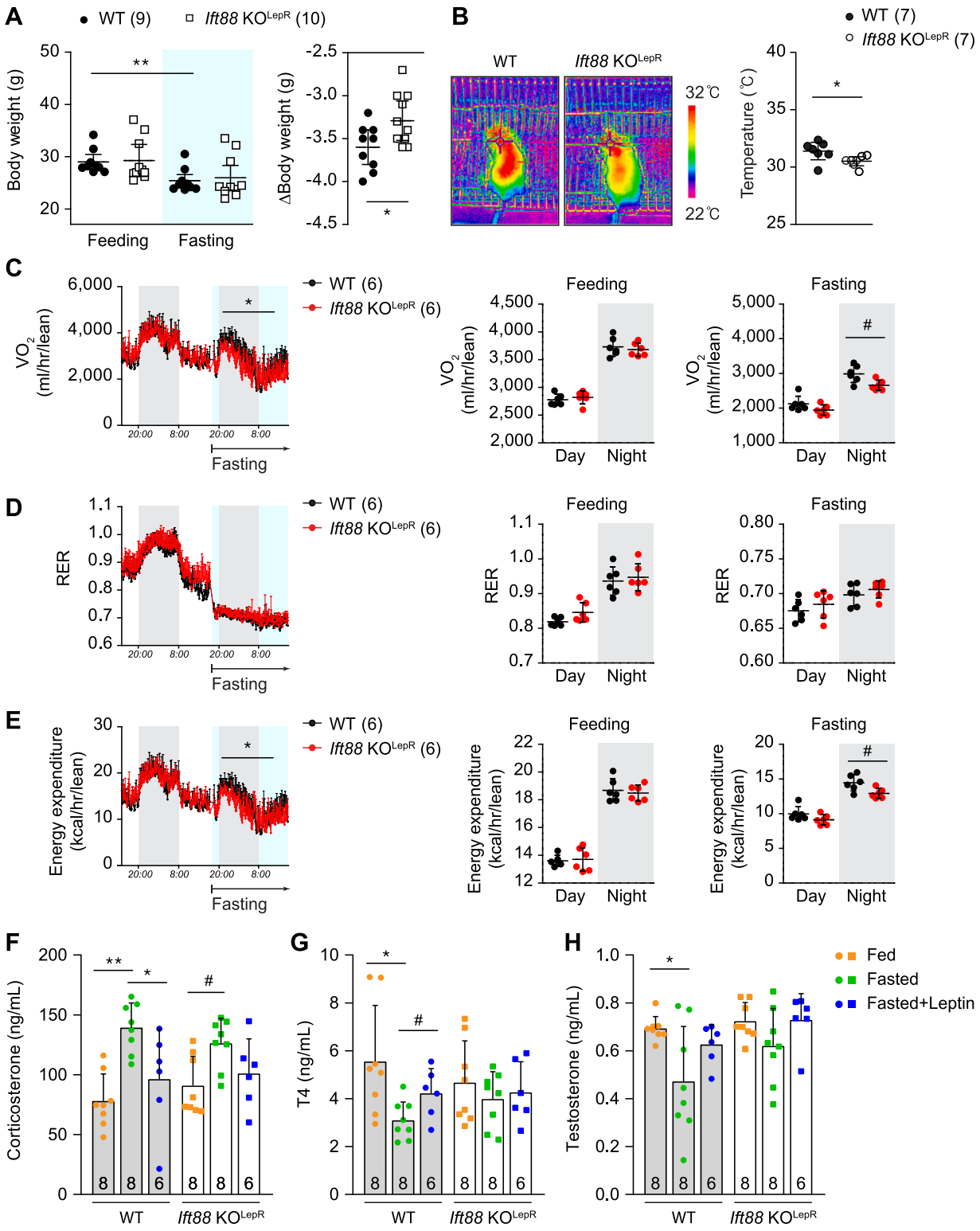

Fig. 2. Primary cilia in LepR neurons are necessary for counterregulatory fasting responses and neuroendocrine reflexes.
(A) Left, Body weight of male littermates before and after 24 h fasting. **p = 0.0043, Student's *t*-test. Right, Δ Body weight. *p = 0.0261, Student's *t*-test.
(B) Left, Thermography images of mice after 24 h fasting. Right, Interscapular skin temperature analysis. *p = 0.0352, Student's *t*-test.
(C to E) Metabolic parameters were monitored during feeding and fasting conditions. Changes in (C) VO_2 [significant genotype effect (*p = 0.0125, 2-way ANOVA. #p = 0.0194, Student's *t*-test)]. (D) RER, and (E) energy expenditure [significant genotype effect (*p = 0.0262, 2-way ANOVA. #p = 0.0207, Student's *t*-test)] were shown.
(F to H) Level of counterregulatory hormones in response to fasting with or without leptin in WT and *Ift88* KO^{LepR} mice. (F) Serum corticosterone (**p = 0.0011, *p = 0.0338, and #p = 0.0295, one-way ANOVA), (G) T4 (*p = 0.0197 and #p = 0.0497, one-way ANOVA), and (H) testosterone (*p = 0.0241, one-way ANOVA) were measured in the indicated conditions.
The number of mice used was expressed in parenthesis. Data are expressed as mean ± SD.

condition using whole-cell patch-clamp technique (Fig. 1B–E and Supplementary Fig. 2B). Bath perfusion of leptin induced mixed responses in WT neurons, with a subset (9 of 24, 37.5 %) of neurons being hyperpolarized at −5.79 ± 2.60 mV whereas a smaller population of the neurons (6 of 24, 25 %) being depolarized at 5.49 ± 3.13 mV (Fig. 1C and E). However, in the *Ift88* KO^{LepR}, only 5 of 21 cells (23.81 %) were hyperpolarized without any depolarizing neurons (Fig. 1D and E). These results demonstrated that primary cilia in LepR neurons are required for proper leptin responses in fasting condition.

3.3. Primary cilia in LepR neurons are essential for adaptive neuroendocrine responses to starvation

Prompt decrease of leptin is a prerequisite condition for normal adaptive responses to starvation [12]. The disrupted leptin homeostasis in fasted *Ift88* KO^{LepR} led us to examine the adaptive counterregulatory responses. The *Ift88* KO^{LepR} displayed significantly reduced weight loss (Δ body weight) and lower body temperature after starvation (Fig. 2A and B). In addition, the *Ift88* KO^{LepR} also presented significantly reduced VO_2 and EE in fasting condition without changing RER (Fig. 2C–E). This highlighted that primary cilia in LepR neurons are mandatory for normal response to starvation. Furthermore, we examined the representative hormones known to play key roles in regulation of hypothalamus-pituitary-adrenal (HPA), -thyroid (HPT), and -gonad (HPG) axes [2,13,14] (Fig. 2F–H). In the case of WT, T4 and testosterone were significantly decreased during starvation (44 % for T4 and 32 % for testosterone, respectively). However, the *Ift88* KO^{LepR} showed only a 14 % decrement for both T4 and testosterone levels (Fig. 2G and H). Unlike WT, the *Ift88* KO^{LepR} exhibited no evident effect of leptin replenishment on those hormones (37 % and 33 % for T4 and testosterone, respectively, in the WT; 7 % and 17 % for T4 and testosterone, respectively, in the *Ift88* KO^{LepR}). In addition, corticosterone levels in the WT showed a 79 % surge by fasting but *Ift88* KO^{LepR} only exhibited an increase of 37 % from the basal level (Fig. 2F). Similar with T4 and testosterone, the effect of leptin on corticosterone was not evident in the *Ift88* KO^{LepR} (31 % vs. 19 % in the WT and *Ift88* KO^{LepR}, respectively). These results clearly suggested that primary cilia, at least in LepR neurons, are essential for proper counterregulatory hormonal responses to starvation.

4. Discussion

Prompted by functional relationship between primary cilia and leptin together with the roles of leptin in fasting condition [2,4,11], we generated primary cilia deletion in LepR neurons and examined their functional roles in modulating adaptive responses.

The *Ift88* KO^{LepR} exhibited a set of impaired counterregulatory responses such as lesser body weight loss, decreased EE, lower heat generation, and higher levels of serum leptin. These results led us to think that primary cilia in LepR neurons may play an important role in the regulation of counterregulatory adaptation by integrating proper leptin action. Indeed, the patch-clamp studies clearly showed that the acute effect of leptin on the LepR neurons of the arcuate nucleus (ARC) in *Ift88* KO^{LepR} was different from the WT. The LepR neurons in WT were either depolarized or hyperpolarized by the exogenous leptin application. However, the LepR neurons with defected primary cilia were only hyperpolarized, indicating that primary cilia are essential for the leptin-mediated neuronal excitability in the ARC. The ARC is consisted of heterogeneous neuronal populations which respond differently to leptin [15]. Therefore, the acquisition of cell type information using novel approaches such as patch-sequencing would be important to delineate the role of primary cilia in adaptive responses.

Prompt decrease of leptin is necessary to activate adaptive neuroendocrine responses such as HPA, HPT, and HPG axes [1,3,12,16]. Expectedly, the WT showed normal hormonal responses under fasting or leptin-replenished conditions. However, these responses were significantly blunted both in the fasting and leptin-replenished conditions in the *Ift88* KO^{LepR}. In addition, the *Ift88* KO^{LepR} after fasting showed a lesser decrease in glucose levels ($p = 0.059$). This result also confirmed the impaired adaptive responses in *Ift88* KO^{LepR} (Supplementary Fig. 3A). Usually, higher T4 and testosterone linked to higher EE. Interestingly, the *Ift88* KO^{LepR} animals showed a lesser decrease in T4 and testosterone whereas their EE were more decreased than WT in fasting condition. This phenotype may come from the central leptin resistance in fasting condition. Central leptin resistance of *Ift88* KO^{LepR} would lead to more EE decrement compared to WT. In addition, the higher leptin levels in fasting condition may act as an inhibitory signal for fasting-induced suppression of the HPT and HPG axes in *Ift88* KO^{LepR}. This subsequently resulted in lesser decrease in T4 and testosterone than WT. Moreover, LepR and primary cilia exist in the pre-adipocytes, therefore, these features may influence the phenotypes [17,18]. Further evaluations on other hormonal levels such as T3, rT3, GH, and IGF-1 would be useful in delineating the functional roles of primary cilia in modulating counterregulatory hormonal responses. To the best of our knowledge, there is yet no report regarding whether a ciliopathy patient has an impaired counterregulatory hormonal responses. Therefore, understanding the mechanisms of how primary cilia regulate hormonal homeostasis could be a therapeutic target for human ciliopathies.

In summary, our current study demonstrate that primary cilia expressed in LepR neurons is a key neuronal component that are required for normal fasting and counterregulatory hormonal responses as well as leptin homeostasis in energy-depleted condition.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2022.155273.

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CRediT authorship contribution statement

Dong Joo Yang: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft, Funding acquisition, Writing – review & editing. **Le Trung Tran:** Investigation, Formal analysis, Writing – review & editing. **Seul Gi Yoon:** Data curation. **Je Kyung Seong:** Resources, Data curation. **Dong Min Shin:** Project administration. **Yun-Hee Choi:** Conceptualization, Funding acquisition, Project administration. **Ki Woo Kim:** Conceptualization, Resources, Supervision, Writing – original draft, Funding acquisition, Writing – review & editing, Project administration.

Declaration of competing interest

All authors declare no competing financial or other interests in relation to this study.

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