News Release

Researchers discovered the mechanism of middle-aged obesity : A major step toward innovative prevention and treatment of lifestyle-related diseases

Key Points

- **The researchers discovered that the melanocortin-4 receptor (MC4R), which has anti-obesity function, is localized to primary cilia of neurons in the hypothalamus of the rat brain, and these primary cilia shorten with age.**
- **The shortening of MC4R-bearing primary cilia caused a decrease in metabolism and an increase in food intake, resulting in obesity.**
- **The length of MC4R-bearing primary cilia determines the susceptibility to obesity, and their shortening due to aging and overnutrition is a cause of age-related obesity (middle-aged obesity).**
- **This discovery is expected to lead to the development of preventive methods and innovative treatments for lifestyle-related diseases at a pre-symptomatic stage.**

Summary

A research group led by Assistant Professor Manami Oya, Senior Lecturer Yoshiko Nakamura, and Professor Kazuhiro Nakamura at the Department of Integrative Physiology, Nagoya University Graduate School of Medicine, in collaboration with Assistant Professor Yoshiki Miyasaka at the Institute of Experimental Animal Sciences, Graduate School of Medicine, Osaka University, Professor Tomoji Mashimo at the Institute of Medical Science, The University of Tokyo, and Senior Lecturer Miyako Tanaka and Professor Takayoshi Suganami at the Research Institute of Environmental Medicine, Nagoya University, has discovered a central mechanism that causes age-related obesity (middle-aged obesity) in rats.

Although people become more susceptible to obesity as they age, the mechanism of this phenomenon was unknown. The research group focused on neurons in the hypothalamus that regulate metabolism and feeding, and investigated how the subcellular localization of the melanocortin-4 receptor (MC4R), which has an anti-obesity function, changes as rats age. The researchers generated the world's first reliable antibody capable of visualizing MC4R proteins and found that MC4Rs localize to the antenna-like structure of primary cilia in hypothalamic neurons and that these primary cilia shorten with

age. Furthermore, the shortening of MC4R-bearing primary cilia was accelerated by overnutrition and inhibited by dietary restriction.

Forced shortening of MC4R-bearing primary cilia in young rats using genetic engineering resulted in increased food intake and decreased metabolic rate, leading to obesity. They also exhibited leptin resistance, which often occurs in obese human patients. Conversely, artificial inhibition of the age-related shortening of MC4R-bearing primary cilia reduced weight gain.

Based on these results, the research group concluded that age-related obesity is caused by a decrease in MC4Rs due to age-related shortening of MC4R-bearing primary cilia in hypothalamic neurons. The results of this study are expected to lead to the development of preventive methods and innovative treatments for various lifestyle-related diseases, such as diabetes, caused by obesity at a pre-symptomatic stage.

Research Background

Obesity has become a major health problem because it leads to various lifestyle-related diseases, such as diabetes and hypertension. Particularly in today's world, where Western-style high-calorie diets are widespread and people are increasingly food-saturated, it is an urgent issue to elucidate the pathogenic mechanism of age-related obesity (so-called middle-aged obesity). Previous studies have suggested that age-related obesity is caused by a decline in whole-body metabolism with age, but the cause and mechanism of the age-related metabolic decline have not been understood.

The research group focused on the melanocortin-4 receptor (MC4R), which is expressed in the hypothalamus of the brain to regulate metabolism and feeding against obesity. When fat accumulates in the body, white adipocytes secrete a hormone called leptin, which acts on the hypothalamus (Figure 1, a). The MC4R, present on neurons in the hypothalamus, receives melanocortin (a satiety signaling molecule) secreted by the action of leptin (Figure 1, b) and activates the firing activities of these neurons, thereby activating neural circuits to increase metabolic rate and heat production (fat burning) and to reduce food intake for an anti-obesity effect (Figure 1, c).

Since mice lacking the MC4R show severe obesity, the MC4R plays a pivotal role in the anti-obesity mechanism. The research group generated the world's first reliable antibody that can visualize MC4R proteins and analyzed how the intracellular localization of MC4R proteins changes with age in the rat hypothalamus.

Research Results

The research group first investigated the location of MC4R proteins in the rat brain using the MC4R antibody they generated. They found that MC4Rs are present only in the hypothalamus, where MC4Rs localize to antenna-like structures called primary cilia of neurons in the paraventricular hypothalamic nucleus and the dorsomedial hypothalamus (Figure 2).

To examine how MC4R localization changes with age, the research group observed the brains of rats at different ages, and found that MC4R-bearing primary cilia gradually shorten with age after 3 weeks of age, when rats are weaned (Figure 3, chow diet). In contrast, primary cilia without MC4Rs did not shorten. Next, they analyzed rats reared under different nutritional conditions and found that age-related shortening of MC4R-bearing primary cilia was accelerated in rats reared on a high-fat diet, whereas it was suppressed by dietary restriction (Figure 3). Furthermore, even MC4R-bearing primary cilia that once disappeared with age were regenerated by dietary restriction.

Therefore, using genetically modified rats (MC4R-Cre knock-in rats) and adeno-associated virus, the research group selectively shortened MC4R-bearing primary cilia in hypothalamic neurons of young rats, and found that the sensitivity to melanocortin, a satiety signaling molecule, was reduced, resulting in decreased metabolic rate and heat production (fat burning) and increased food intake, which led to a marked increase in body weight and body fat percentage compared to control rats (Figure 4). Conversely, inhibiting age-related shortening of MC4R-bearing primary cilia using a similar genetic technique reduced weight gain.

Next, they sought to elucidate the mechanism of shortening of MC4R-bearing primary cilia. First, they analyzed Zucker fatty mutant rats with attenuated leptin-mediated satiety signaling. Since these rats were severely obese, it was initially expected that shortening of MC4R-bearing primary cilia would progress, but unexpectedly, the shortening was inhibited. This suggested that melanocortin itself, whose secretion is stimulated by the action of leptin, may promote age-related shortening of MC4R-bearing primary cilia. Therefore, the researchers chronically administered leptin or continuously stimulated melanocortin signaling in hypothalamic neurons using a genetic technique in wild-type rats, and found that these manipulations accelerated age-related shortening of MC4R-bearing primary cilia. These results indicate that chronic leptin action allows melanocortin to continuously act on MC4Rs on the hypothalamic neurons, thereby accelerating the shortening of MC4R-bearing primary cilia (Figure 5).

More interestingly, leptin, which is secreted by white adipocytes, exerts its

anti-obesity effect by reducing food intake, but rats with shortened MC4R-bearing primary cilia exhibited “leptin resistance”, in which they did not eat less after leptin administration. Leptin resistance, in which leptin is ineffective and has no anti-obesity effect, is often observed in obese human patients. The cause of this phenomenon has long been unknown and has been a major problem in the treatment of obesity. The results of this study showed that leptin resistance is caused by the shortening of MC4R-bearing primary cilia, which is driven by the chronic action of melanocortin triggered by leptin, which is secreted in large amounts by the accumulated white adipocytes in obese patients.

Research Summary and Future Perspective

The research group discovered that MC4Rs are localized in the primary cilia of hypothalamic neurons that regulate metabolism and feeding, and that these primary cilia shorten with age. They also revealed the mechanism of age-related obesity, in which age-related shortening of MC4R-bearing primary cilia decreases MC4Rs present on the neurons and thereby decreases the neuronal sensitivity to melanocortin, resulting in decreased metabolic rate and increased food intake to develop obesity and leptin resistance (Figure 5). In other words, the length of MC4R-bearing primary cilia determines the susceptibility to obesity and their shortening due to aging and overnutrition leads to obesity.

Although the short-term action of melanocortin is anti-obesity, the results of this study indicate that the chronic action of melanocortin on MC4Rs on neuronal primary cilia under continued overnutrition promotes age-related shortening of MC4R-bearing primary cilia in hypothalamic neurons, placing animals in a “negative spiral toward obesity” in which melanocortin gradually becomes ineffective. As obesity progresses, blood leptin levels increase (hyperleptinemia) and leptin resistance develops. The results of this study suggests that this may be due to the fact that high leptin concentrations chronicize the effects of melanocortin and shorten MC4R-bearing primary cilia in hypothalamic neurons, resulting in the loss of its anti-obesity effects.

Human patients with Bardet-Biedl syndrome, a genetic disorder with defective or dysfunctional primary cilia, exhibit severe obesity, and therefore, defective or degenerated primary cilia is linked to obesity in humans as well as rats. Given the results of this study, it is likely that the loss of MC4R-bearing primary cilia in hypothalamic neurons is a cause of the obesity in Bardet-Biedl syndrome. To investigate the mechanism of age-related obesity in humans, further research is needed to verify whether the age-related shortening of

MC4R-bearing primary cilia found in rats in this study also occurs in humans.

This study discovered age-related shortening of primary cilia for the first time in the world. Age-related shortening may also occur in other primary cilia, and it may be a cause of various diseases. In the future, by elucidating the molecular mechanism by which the structure of primary cilia changes with age, it is hoped that this research will be extended to the development of drugs that prevent shortening. In addition, the fact that primary cilia with MC4Rs shorten to reduce their sensitivity to satiety signals is thought to have an important physiological significance, and basic research to elucidate this is also needed in the future.

In this study, the research group also presents methods to inhibit the shortening of MC4R-bearing primary cilia, such as dietary restriction and genetic manipulation. In particular, the observation that MC4R-bearing primary cilia once lost due to aging were regenerated by dietary restriction is a finding that offers great hope for the treatment of obesity. Obesity is a condition that serves as a gateway to several lifestyle-related diseases, such as diabetes. The research group hopes to use the findings to develop technologies to prevent the onset of lifestyle-related diseases at a pre-symptomatic stage, and to develop innovative treatments for obesity.

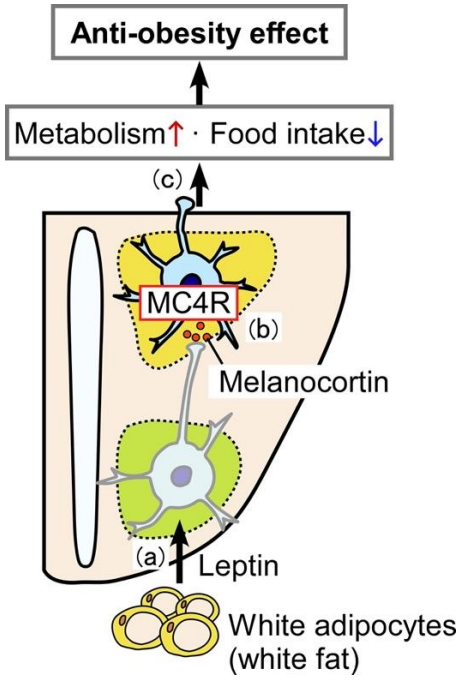

Figure 1: The MC4R acts in the hypothalamus to prevent obesity by receiving satiety signals.

Leptin, secreted by white adipocytes (white fat cells), acts on the hypothalamus (a) to induce the secretion of melanocortin, a satiety signaling molecule. The secreted melanocortin is received by the MC4R located on neurons (b), and then the activated receptor induces the excitation of the neurons to activate neural circuits that promote metabolism and suppress feeding for an anti-obesity effect (c).

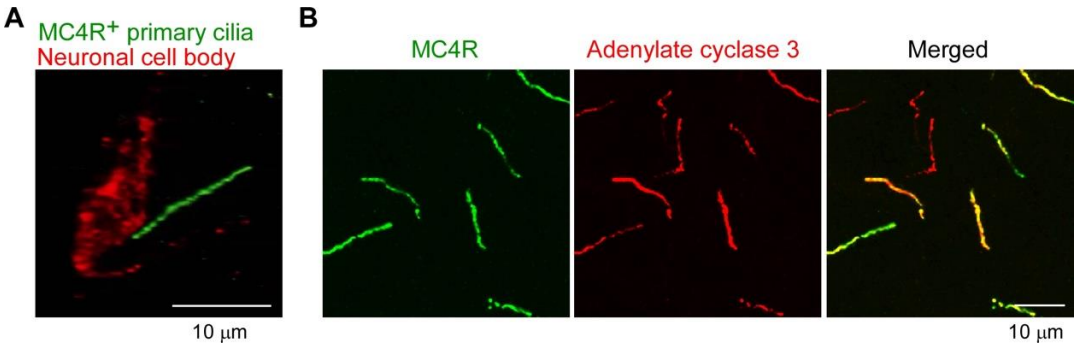

Figure 2: MC4Rs localize to primary cilia of hypothalamic neurons.

(A) An MC4R-bearing primary cilium (green) protruding from a hypothalamic neuronal cell body (red). (B) MC4R proteins in the hypothalamus were labeled green and adenylate cyclase 3 was labeled red. Adenylate cyclase 3 is known to localize to primary cilia of neurons and was used as a marker for primary cilia. MC4Rs were found to localize to primary cilia (yellow when merged).

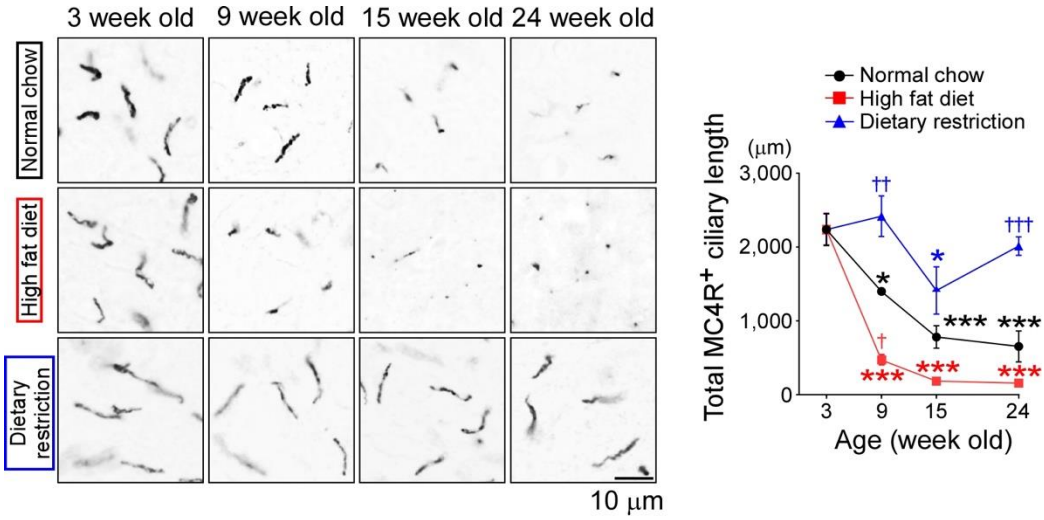

Figure 3: MC4R-bearing primary cilia of hypothalamic neurons shorten with age.
MC4R-bearing primary cilia of hypothalamic neurons shortened with age (normal chow). Rats fed a high-fat diet shortened faster than rats fed a normal chow. Age-related shortening was suppressed in rats whose food intake was restricted to 60% of that of normal chow rats. Two-way ANOVA followed by Bonferroni's *post hoc* test, $*p < 0.05$, $***p < 0.001$ (*vs* 3 weeks old); $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ (*vs* Normal chow).

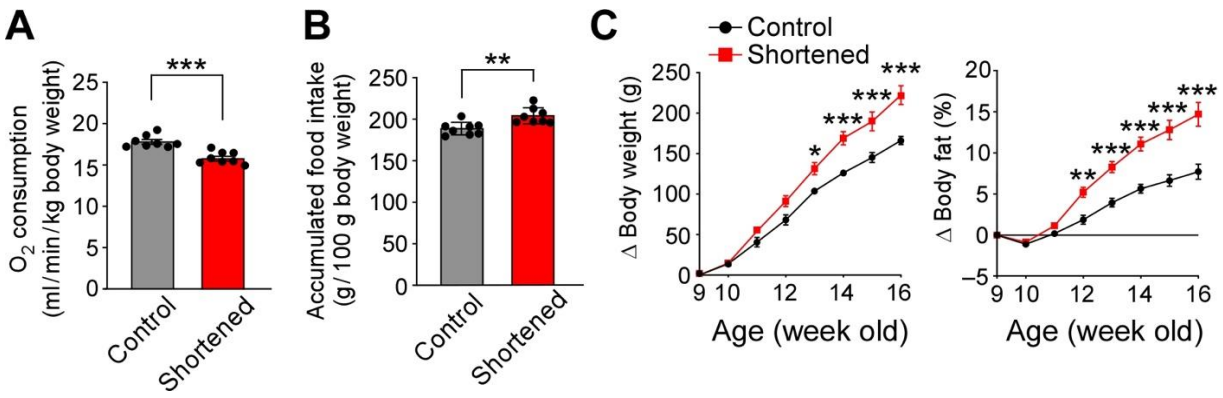

Figure 4: Forced shortening of MC4R-bearing primary cilia of hypothalamic neurons causes obesity.
Rats with genetically shortened MC4R-bearing primary cilia of hypothalamic neurons (Shortened) consumed less oxygen (meaning lower metabolic rate) (A) and ate more (B) than control rats. As a result, the shortened rats showed a significantly greater increase in body weight and body fat percentage than the control rats (C). Two-way ANOVA followed by Bonferroni's *post hoc* test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (*vs* Control).

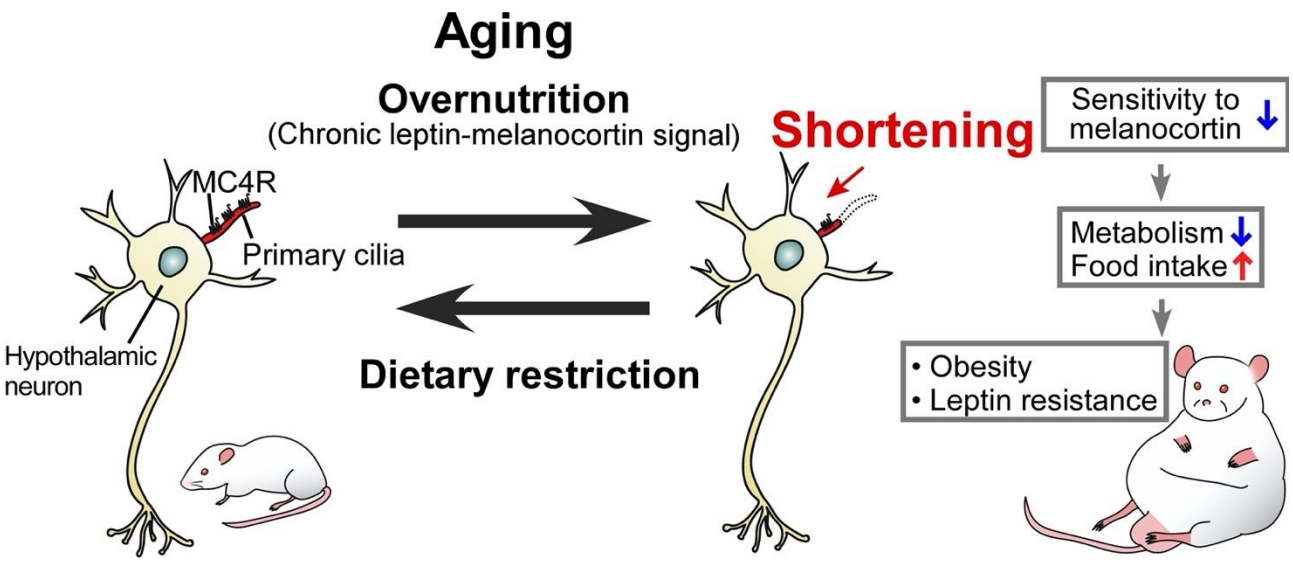

Figure 5: Mechanism of the development of age-related obesity.
Aging shortens MC4R-bearing primary cilia of hypothalamic neurons. In addition, chronic leptin-melanocortin signaling, exacerbated by overnutrition such as high-fat diets, promotes their shortening. The shortening of MC4R-bearing primary cilia reduces the neuronal sensitivity to melanocortin, a satiety signaling molecule, and thereby leads to decreased metabolic rate and increased food intake, resulting in obesity and the development of leptin resistance. Dietary restriction inhibits the shortening of MC4R-bearing primary cilia.

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