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Voluntary exercise contributed to an amelioration of abnormal feeding behavior, locomotor activity and ghrelin production concomitantly with a weight reduction in high fat diet-induced obese rats

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ABSTRACT

In the present study, effects of voluntary exercise in an obese animal model were investigated in relation to the rhythm of daily activity and ghrelin production. Male Sprague–Dawley rats were fed either a high fat diet (HFD) or a chow diet (CD) from four to 16 weeks old. They were further subdivided into either an exercise group (HFD-Ex, CD-Ex) with a running wheel for three days of every other week or sedentary group (HFD-Se, CD-Se). At 16 weeks old, marked increases in body weight and visceral fat were observed in the HFD-Se group, together with disrupted rhythms of feeding and locomotor activity. The induction of voluntary exercise brought about an effective reduction of weight and fat, and ameliorated abnormal rhythms of activity and feeding in the HFD-Ex rats. Wheel counts as voluntary exercise was greater in HFD-Ex rats than those in CD-Ex rats. The HFD-obese had exhibited a deterioration of ghrelin production, which was restored by the induction of voluntary exercise. These findings demonstrated that abnormal rhythms of feeding and locomotor activity in HFD-obese rats were restored by infrequent voluntary exercise with a concomitant amelioration of the ghrelin production and weight reduction. Because ghrelin is related to food anticipatory activity, it is plausible that ghrelin participates in the circadian rhythm of daily activity including eating behavior. A beneficial effect of voluntary exercise has now been confirmed in terms of the amelioration of the daily rhythms in eating behavior and physical activity in an animal model of obesity.

1. Introduction

Obesity has been recognized as a serious burden all over the world, in both developing and developed countries. Worldwide, the proportion of adults with a body mass index (BMI) of 25 kg/m^2 or greater was estimated as more than 35% in both genders and is still increasing every year [1]. Food restriction and regular exercise are the two major requirements for the treatment of obesity and metabolic syndrome, which are coupled with insulin resistance and cause life style related disease, such as diabetes, hypertension and dyslipidemia. However, obesity is often associated with physical inactivity and disrupted life rhythms, including binge and night eating [2], which makes the treatment of obesity more complicated and weight reduction less attainable. Although exercise is recommended for the purpose of weight reduction from the aspect of energy expenditure, it is generally difficult for most obese subjects to continue regular exercise for a long duration.

Ghrelin, originally identified as a growth hormone secretagogue (GHS), is an orexigenic gut hormone. It is a 28 amino acid peptide produced by the X/A-like endocrine cells in the oxyntic glands of the gastric fundus [3,4]. Ghrelin functions primarily as an orexigen [5] and as a GH-releasing hormone [3]. Various

Abbreviations: CD, chow diet; FAA, food anticipatory activity; GHS, growth hormone secretagogue; GOAT, ghrelin O – acyltransferase; HFD, high fat diet.

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other physiological roles have been reported, including modulation of energy metabolism [6], and regulation of the autonomic nervous system [7,8] and cardiovascular system [9]. The biological activities of ghrelin require octanoylation of the peptide on Ser_3, an unusual post translational modification that is catalyzed by the enzyme ghrelin O – acyltransferase (GOAT) [10,11]. Although the circulating ghrelin levels are decreased in obese human subjects [12,13], the obese Zucker rat is characterized by increases in the plasma ghrelin concentration, as well as the stomach ghrelin mRNA expression [14], indicating that the relationship between ghrelin synthesis and the progression of obesity is complex and not fully elucidated. In contrast to a significant decrease after a single bout of exercise [15], the long-term outcomes of periodic exercise on the ghrelin production are still controversial and have not been clarified. A 12-months aerobic exercise program was associated with increased total ghrelin concentrations in overweight or obese postmenopausal females [16], while three weeks of moderate treadmill exercise reduced the plasma and fundus total ghrelin concentrations in male rats [17], and a three-week multidisciplinary weight-reduction intervention involving individualized physical activity in obese adolescents did not affect the ghrelin levels produced in response to meals and exercise [18].

Some earlier studies suggested that GHSs induce phase advances in the circadian rhythm, indicating a role for GHSs in circadian physiology [19,20]. The administration of ghrelin in the absence of food increases locomotor activity and subsequent food intake, known as food anticipatory activity (FAA). In the absence of ghrelin receptors, this food anticipatory behavior is diminished [21], thus suggesting a crucial role of ghrelin in FAA. Therefore, it has been hypothesized that ghrelin participates in the homeostasis of energy metabolism and the circadian rhythm of daily activity, including eating behavior. The aim of the present study was to investigate the effects of voluntary exercise on the disrupted rhythms of daily activity, which are often observed in obese subjects, in relation to the amelioration of extraordinary ghrelin production in a high fat diet (HFD)-induced obese model.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats (Jcl:SD, CREA Co Ltd., Osaka, Japan) were used in this study. The animals were housed in a controlled room (temperature 25 ± 2 °C, humidity 60 ± 10%) under a 12 h light–dark cycle (light on 0700–1900) with ad libitum access to either control chow diet (CD; 10 kcal% fat, produced by Research Diets, Inc., New Brunswick, NJ, USA: open source diet code D12450B) or high fat diet (HFD; 60 kcal% fat, produced by Research Diets, Inc., New Brunswick, NJ, USA: open source diet code D12492) and water.

2.2. Experimental protocols

Rats at four weeks old were divided into four groups: sedentary groups fed either CD (CD-Se, $n = 6$) or HFD (HFD-Se, $n = 6$) and exercise groups fed either CD (CD-Ex, $n = 6$) or HFD (HFD-Ex, $n = 6$). For habituated period, all rats were individually housed in ordinary clear plastic TPX® cages (W27 cm × D43 cm × H20 cm) with paper bedding for 11 days. For the measurement of respiratory gas and locomotor activity, they were moved into specially designed acrylic metabolic chambers equipped with a running wheel apparatus (36 cm diameter and 11.8 cm width) for three days of every 2nd weekend. These programs for 2 weeks were repeated from 6 to 16 weeks old as illustrated in Fig. 1. Rats in exercise groups (CD-Ex and HFD-Ex) could freely access to the wheel and ran on wheel voluntarily for three days. Rats in sedentary groups (CD-Se and HFD-Se) were prevented from accessing the wheel by an interception board. All the experiments were performed in accordance with protocols approved by the Kurume University Animal Experiment Committee, based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1996).

2.3. Measurement of body weight, food intake, and fat distribution

Body weight of rats as well as food intake during either light or dark period were measured every other week from 4 to 16 weeks old. A saucer had been equipped beneath each feeder, and food spill was collected and measured. Food intake was then calculated subtracting this spilt food from the intake value. Visceral and subcutaneous fat volumes (mm^3) were measured at 16 weeks old using in vivo micro-computed tomography (R_mCT2, Rigaku Co., Tokyo, Japan) under imaging conditions of FOV73 (φ 73 mm × H57 mm), 90 kV tube voltage and 160 μA tube current. Rats were anesthetized with 3% isoflurane (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and placed supine in the machine, and serial 4 mm scans were performed from the anterior to the posterior aspect of lumbar vertebra 4. Fat analysis software (Rigaku Co., Tokyo, Japan) estimated the volumes of adipose tissue, bone, air and the remainder on the basis of their different X-ray densities, and distinguished

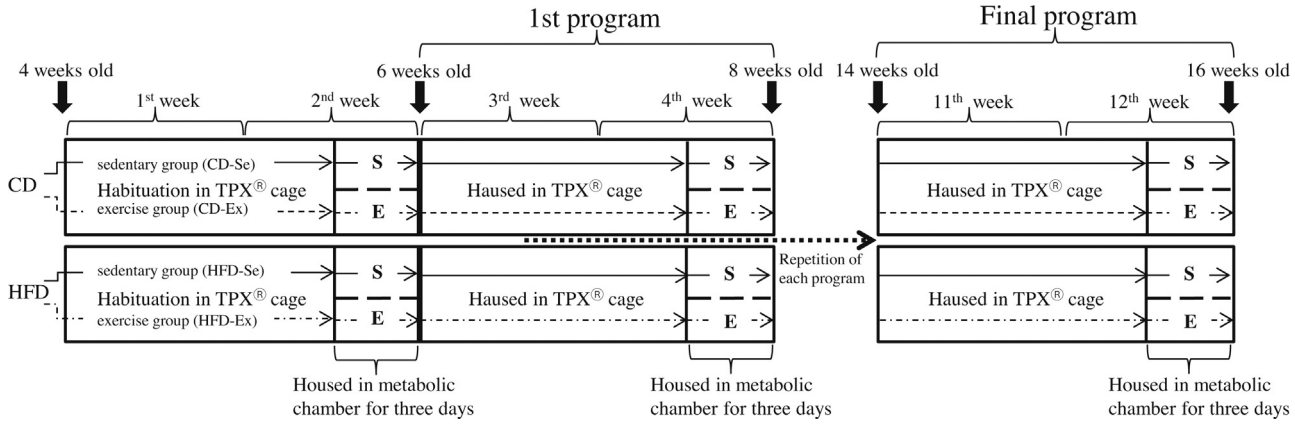

Fig. 1. Experimental sedentary and exercise program. All rats were individually housed in ordinary clear plastic TPX® cages for 11 days. For the measurement of respiratory gas and locomotor activity, they were moved into specially designed acrylic metabolic chambers equipped with a running wheel apparatus for three days of every 2nd weekend. These programs for 2 weeks were repeated from 6 to 16 weeks old. S, rats were prevented from accessing a wheel by an interception board; E, rats could freely access to a wheel and ran on it voluntarily.